



BLCA-4 IN CYSTITIS AND SCHISTOSOMAL CHRONIC CYSTITIS

Huda Sadoon Jassim Al-Biaty

Department of parasitology, College of Vet. Med., University of Baghdad

*Corresponding authors email: huda.sadoon@yahoo.com

ABSTRACT

Bladder Cancer Associated Protein-4 (BLCA-4) is a nuclear transcription factor functioning in the regulation of the gene expression related to cell growth. The aim of this study is to evaluate the level of urinary BLCA-4 in cystitis (Schistosomal and non Schistosomal). Bladder biopsies and urine samples were collected from 50 cystitis patients (Schistosomal chronic cystitis and non Schistosomal cystitis) also from 38 healthy individuals as control. Concentration of BLCA-4 was calculated by sandwiches ELISA, sensitivity and specificity were measured also. The result showed no significant increase of BLCA-4 level in cystitis than healthy, as well as no significant difference in mean level of BLCA-4 among different age, sex of patients. Both Schistosomal chronic cystitis and non-Schistosomal cystitis patients were with no significant difference in BLCA-4 mean level. BLCA-4 was with sensitivity (91.023%) and specificity (78.127%) in cystitis patients. We conclude that ELISA test for urine BLCA-4 showed low level in cystitis.

KEYWORDS: BLCA-4, Cystitis, Schistosoma, Urine, Inflammation, Histopathology.

INTRODUCTION

For the first time nuclear matrix structure was described by Berezney and Coffey. Then McCready *et al.*, 1980 found that this protein component was a raised from triple structural regions first a lamina containing nuclear pores, then nucleoli residual, lastly framework of an internal matrix which is connected to a residual nuclear layer that contain complex pore. Later on, six bladder-specific nuclear structure proteins (BLCA-1 to 6) were identified by (Getzenberg *et al.*, 1996), these nuclear structure proteins were expressed exclusively by bladder cancer cells (Konecy and Getzenberg, 1999).

In 2004, genes sequencing encode BLCA-4 were demonstrated by (Van Le *et al.*, 2004). These genes sequencing (E-transformation-specific, ETS) are transcription factors involved in expression of vascular endothelial growth factor (VEGF), apoptosis, angiogenesis, carcinogenesis (Oikawa, 2004). Also BLCA-4 reacts with many transcription factors, such as Nuclear factor of activated T-cell (NFATc), Activating protein -1(AP-1, 2) and Nuclear -factor erythroid- 1(NF-E1, 2), (Van Le *et al.*, 2004), that revealed an up-regulation of cell growth genes, like cyclins, TM (thrombomodulin), Interleukin- 1 -alpha (IL-1) and Interleukin -8(IL-8) with the analysis of gene promoters of them their sequence was with multiple ETS sites suggesting the direct binding of BLCA-4 to each gene in order to cause over expression (Myers-Irvin *et al.*, 2014). In patients with different benign urologic disorders such as catheterization, infection of urinary tract and schistosomal chronic cystitis which consider the second parasitic disease after malaria (WHO, 2014), BLCA-4 high level was absence (Santoni *et al.*, 2012).

MATERIALS & METHODS

From August 2015 to January 2016 in AL-Yarmouk teaching hospital, Baghdad-Iraq, tissue samples of inflamed bladder were obtained by cystoscopy from 50 patients 36 (72.00%) cases were males, 14 (28.00%) cases were females with pathologically confirmed cystitis, blocks of tissues were prepared for microscopic examination by fixation in 10% neutral formalin and then dehydrated in ethanol alcohol, embedded in paraffin wax, cut at thickness of 3-4 μ m, and then stained with hematoxylin and eosin (H-E) for examination under a light microscope (Suvarna *et al.*, 2013) and the pathological type was divided to schistosomal related cystitis and non-schistosomal cystitis. schistosoma diagnosis was based on the finding calcified eggs in tissues biopsies, urine BLCA 4 protein was analyzed by sandwiches ELISA (abcam, UK) in those 50 cystitis patients and in 38 normal individuals by centrifuged at 3000 RPM for 10 min., and the supernatant was stored at -80°C until immunological analysis. Urine BLCA4 concentrations in samples were determined by comparing the optical densities (OD) of the samples with the standard curves.

Sensitivity and specificity of BLCA-4 as a marker were determined by ROC analysis

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Least significant difference -LSD or T-test was used to significant compare between means, and estimate of Sensitivity and Specificity in this study.

RESULTS

Assessment of BLCA-4 in cystitis group:

Urinary BLCA-4 was not significantly higher in the cystitis group (5.76 ng/ml) than healthy (5.61 ng/ml) with P value of 0.23 (Table-1; Figure -1). The level was in no

BLCA-4 in cystitis and schistosomal

relation to gender ($P=0.30$), (Table-2) and age ($P=0.31$), (Table-3), the higher mean level of urine BLCA-4 was in 40-49 age group(5.85ng/ml) and the lower in 30-39 age group (5.37ng/ml).

Schistosomal chronic cystitis patients showed increase in BLCA-4 concentration (5.82ng/ml) than non schistosomal

cystitis patients (5.78ng/ml) but with non-significant difference (Table-4, Figure- 2). According to sex and age, urineBLCA-4 levels showed no significant differences ($p=0.924$) ($P=0.46$) respectively, (Table-5 and Table-6).

TABLE 1: Comparison between cystitis and healthy group in the mean level of urine BLCA-4

BLCA-4(ng/ml)	Cystitis group	Healthy group
No.	50	38
Mean	5.76	5.61
SD	0.64	0.48
SE	0.09	0.07
Minimum	4.93	4.86
Maximum	8.15	7.71
T-test	0.247 NS*	
P-value	0.2327	

NS: Non-significant.

TABLE 2: Urine BLCA-4 level in cystitis patients according to gender

BLCA-4(ng/ml)	Gender	
	Male	Female
No. (%)	36 (72.00%)	14 (28.00%)
Mean	5.73	5.63
SD	0.18	0.22
T-test	0.266 NS*	
P-value	0.306	

NS: Non-significant.

TABLE 3: Urine BLCA-4 levels in cystitis patients according to age groups

BLCA4(ng/ml)	Age (years)				
	30-39	40-49	50-59	60-69	70
No.	3	10	15	18	4
(%)	(6.0%)	(20.0%)	(30.0%)	(36.0%)	(8.0%)
Mean	5.37	5.85	5.63	5.74	5.55
SD	0.28	0.32	0.21	0.18	0.21
LSD value	0.486 NS*				
P-value	0.315				

NS: Non-significant.

TABLE 4: Urine BLCA-4 level between schistosomal chronic cystitis and non-schistosomal cystitis

BLCA-4(ng/ml)	Schistosomal chronic cystitis	Non-schistosomal cystitis
No.	8	42
Mean	5.82	5.78
SD	0.69	0.66
SE	0.09	0.10
Minimum	4.99	4.93
Maximum	8.17	8.15
T-test	0.271 NS*	
P-value	0.859	

NS: Non-significant.

TABLE 5: Urine BLCA-4 level in schistosomal chronic cystitis in regard to sex

BLCA-4(ng/ml)	Gender	
	Male	Female
No. (%)	4 (50.0%)	4 (50.0%)
Mean	5.75	5.82
SD	0.19	0.26
T-test	0.308 NS	
P-value	0.924	

NS: Non-significant.

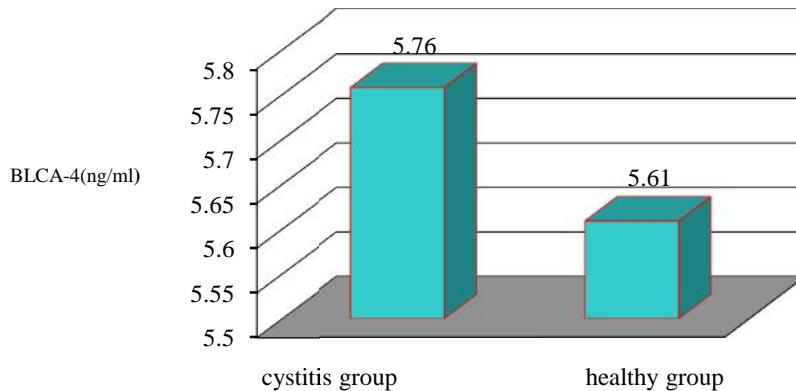
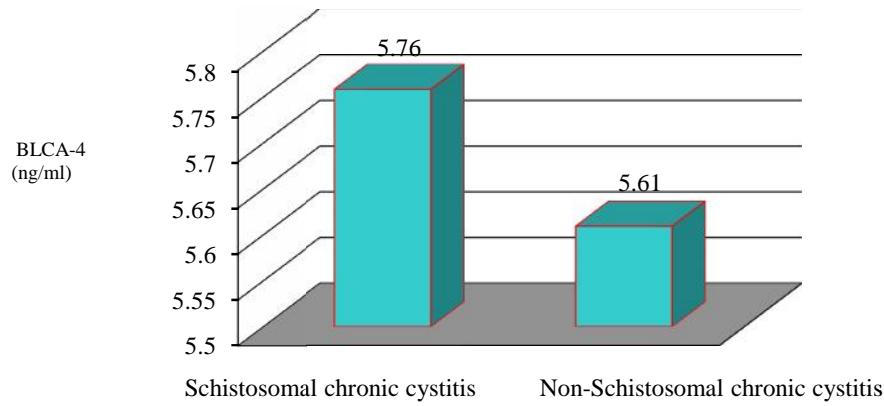
TABLE 6: Urine BLCA-4 level in schistosomal chronic cystitis in regard to age

BLCA-4(ng/ml)	Age (years)		
	40-49	50-59	60
No. (%)	2 (25.0%)	3 (37.5%)	3 (37.5%)
Mean	5.87	5.66	5.79
SD	0.27	0.23	0.23
LSD value	0.336 NS*		
P-value	0.469		

NS: Non-significant.

TABLE 7: The ROC analysis for BLCA-4

BLCA-4	Group	SE	95% CI	Sensitivity (%)	Specificity (%)	P-value
	Cystitis	0.04	0.937-1.00	91.023	78.127	0.029

**FIGURE 1:** Mean urine level of BLCA-4 in cystitis and healthy group**FIGURE 2:** Urine BLCA-4 in Schistosomal chronic cystitis

Receiver Operating Characteristic (ROC) analysis

This test was used to know the accuracy of a any test that divided subjects into diseased or non, so for diagnosis of cystitis BLCA-4 was with specificity of 78.127% for the diagnosis of the disease and sensitivity of 91.023 (Table-7).

DISCUSSION

Recently uBLCA-4 showed a potential utility as a urine marker (Xia *et al.*, 2014).

In this study the result showed no significant increase ($P=0.23$) in BLCA-4 level in cystitis patients (5.76 ng/ml) when compared with healthy (5.61ng/ml), this in agreement with (Santoni *et al.*, 2012) who recorded low

level of urine BLCA-4 in patients with cystitis, while in bladder cancer Al-Biatty,2015 noted significant increase in its level, BLCA-4 may not effect pro-angiogenic pathways but it can however enhance tumorigenesis ,cellular proliferation and tumor invasiveness through interaction with Interleukin -8(IL-8), Interleukin- 1 -alpha (IL-1), Matrix metallopeptidase-9(MMP-9) and vascular endothelial growth factor (VEGF) (Myers-Irvin *et al.*, 2014; Al-Biatty, 2015; Feng *et al.*, 2012). On the other side IL-8 and MMP-9 was increased in cystitis group (Al-Biatty, 2016), Yet BLCA-4 did not show this increase in cystitis group of our recent study. When evaluating the level of urinary BLCA-4 with age and sex no significant correlation between urine BLCA-4 level with patients age

and sex, Similar result was reported by Feng *et al.*,2011 who revealed no statistical correlation between BLCA-4 with age and sex, but a correlation demonstrated between this factor with age and gender by (Konety *et al.*,2000). As well as no statistical correlation was found in present study between BLCA-4 and *Schistosomiasis*, this was similar to (Al-Biaty, 2015). Sensitivity and specificity of urinary BLCA-4 by ELISA tests reached (91.023% and 78.127%) respectively, however Feng *et al.*, 2011 revealed 97.3% sensitivity and 100% specificity in bladder cancer, from these finding besides an absence of high BLCA-4 levels in benign genitourinary conditions, we can use urinary BLCA-4 for the screening of bladder cancer (Van Le *et al.*, 2004; Santoni *et al.*,2012).

CONCLUSION

BLCA-4 in urine measured by sandwiches ELISA was with no significant difference in cystitis patients than normal, schistosomal than non schistosomal chronic cystitis, age and sex. BLCA-4 is not specific (78.127 %) marker for cystitis, and cannot be detected by using a urine-based assay.

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