

Urinary level of Matrix Metalloproteinase (MMP-9) as a biomarker in cystitis and schistosomal chronic cystitis patients

Huda Sadoon Jassim AL-Biaty

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Address for Correspondence:

Huda Sadoon Jassim AL-Biaty,
Department of parasitology, College of
vet. Medicine, University of Baghdad

Abstract

Cystitis means inflammation of the bladder, specifically inflammation of bladder wall. Evaluating the level of urinary MMP-9 as a prognostic biomarker in cystitis (schistosomal and non schistosomal) was the aim of the study. Urine samples and bladder biopsies were collected from 50 cystitis patients who were included schistosomal chronic cystitis and non schistosomal cystitis and also from 38 healthy individuals as control. Urinary concentration of MMP-9 was measured by ELISA technique. Specificity and sensitivity of marker were also calculated. The results showed that MMP-9 urinary level was significantly increased in cystitis patients (3842.90pg/ml) compared to healthy (634.80pg/ml), there was a significant differences in the mean levels of urine MMP-9 for different age groups of patients, but non-significant between females and males. MMP-9 was elevated in both schistosomal chronic cystitis and non-schistosomal cystitis patients and this elevation of marker was higher in schistosomal chronic cystitis but with non-significant, also urine MMP-9 showed significant increase in males than females schistosomal cystitis, and statistical differences between age groups. Matrix Metalloproteinase was specific (87.47%) and was more sensitive (93.22 %) in cystitis patients. We concluded that MMP-9 has a role in inflammation processes of cystitis.

Key words: MMP-9, Cystitis, Schistosoma, Inflammation, Urine, Biopsy

INTRODUCTION

Matrix metalloproteinases which are involved in extracellular matrix degradation (ECM) are a large family of calcium-dependent zinc-containing endopeptidases, MMP-9 has a central role in proliferation of cell, differentiation, migration, angiogenesis, host defenses mechanism and apoptosis (1). It was recorded that deregulation of MMPs has been associated with many conditions like chronic ulcers, encephalomyelitis, arthritis as well as cancer (2), it excretes as an inactive pro-enzyme then undergoes activation by different types of extracellular proteases (3). Activity of MMP-9 regulated by different biochemical stimulators as growth factor and cytokines like (IL-8) in which the pathways of intracellular signaling modulated due to their expression (4). Lee et

al., 2008 showed that tumor necrosis factor-alpha (TNF) stimulated the secretion of pro inflammatory MMP-9 through activation of transcription factors, which are involved in the Extracellular-signal-Regulated kinases (ERK1/2) and Protein(p38)Mitogen-Activated Protein(MAP) kinase mediated control of MMP-9 regulation, namely, Specificity Protein- 1(Sp-1), Activating Protein 1(AP-1) and Nuclear Factor kappa-B (NF-kappaB),(6,7).

There is a relationship between cystitis and neglected tropical disease schistosomiasis (8). With about 600 million people exposed to infection in Asia, Africa, South America schistosoma ranks the second common parasitic disease after malaria (9). Matrix metalloproteinase (MMP-9) has been associated with cystitis related to schistosoma infection (10), and the

initiation of pathological changes, fibrosis found in advanced schistosomiasis was related to MMP-9 (11).

PATIENTS AND METHODS

From August 2015 to January 2016 in AL-Yarmouk teaching hospital specimens of urinary bladder biopsies were collected from the inflammatory lesions of bladder by cystoscopy, fifty patients 36(72.00%) cases were males, 14(28.00%) cases were females were included in this study, 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 μm, and then stained with hematoxylin and eosin (H-E) for examination under a light microscope 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.

As well as voided urine samples were collected from:-

- Those Fifty cystitis patients.
- And from thirty eight healthy volunteers, 25 cases were males, 13 cases were females.

Urinary level MMP-9 was measured by ELISA technique (abcam, UK) for patients and control after centrifuged at 3000 RPM for 10 min, and the supernatant was stored at -80°C until immunological analysis. Urine MMP-9 concentrations in samples were determined by comparing the optical densities (OD) of the samples with the standard curves.

Sensitivity and specificity of MMP-9 as a marker were determined by ROC analysis.

RESULT

MMP-9 in cystitis group:

Urinary level of MMP-9 was significantly elevated in cystitis patients group (3842.90 pg/ml) compared to control (634.80pg/ml) with P value of < 0.001 (Table-1; Figure -1).

Table-1:- Comparison between cystitis and healthy group in the mean level of urine MMP-9

Results	Cystitis group	Healthy group
No.	50	38
Mean (pg/ml)	3842.90	634.80
SD	1285.81	921.03
SE	181.84	149.41
Minimum	16087.10	101.31
Maximum	6257.67	4494.368
T-test	489.100 *	
P-value	0.0001	
*: Highly Sig.		

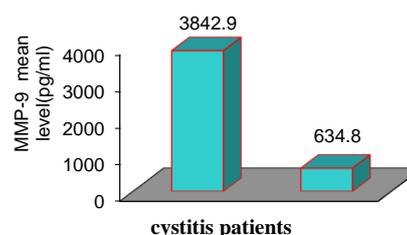


Figure-1:-Comparison between cystitis and healthy group in MMP-9 urine level

There were variations in the mean levels of urine MMP-9 in different age groups of cystitis patients; these variations were significant (Table-2). The higher mean level of urine MMP-9 seems to be in 40-49 age group(4364.38pg/ml) and the lower in 60-69 age group (3463.02pg/ml).

Table -2:- Effect of age groups in urine MMP-9 levels in cystitis patients

Results	Age (years)				
	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	3563.02	4364.38	4043.34	3463.02	3506.47
SD	1351.89	983.24	1454.53	1321.21	1067.92
LSD-value (F)	684.24 *				
P-value	0.0506				
*: (P<0.05) Sig					

In contrast urine MMP-9 was with non -significant difference according to gender (P >0.01),(Table-3)

Table-3:- Effect of gender in MMP-9 level in urine of cystitis patients.

Results	Gender	
	Males	Females
No. (%)	36 (72.00%)	14 (28.00%)
Mean	3941.70	3588.82
SD	1332.16	1165.00
T-test	761.39 NS*	
P-value	0.2774	
* NS: Non-Significant.		

MMP-9 in schistosomal chronic cystitis

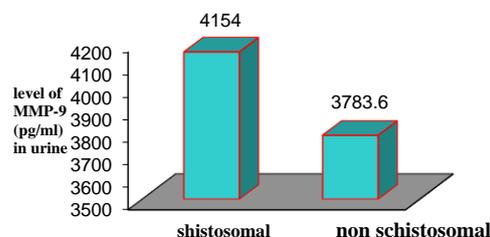


Figure-2:-Schistosomal chronic cystitis and non schistosomal cystitis MMP-9 urine level

MMP-9(pg/ml) showed elevation in urine of schistosomal chronic cystitis patients than non schistosomal cystitis patients

but with non- significant difference(4154.00 vs 3783.60),(Table-4,Figure-2)

Table-4:- Comparison in urine MMP-9 level between schistosomal chronic cystitis and non-schistosomal cystitis

Results	schistosomal chronic cystitis group	Non- schistosomal cystitis group
No.	8	42
Mean	4154.00	3783.60
SD	1530.02	1246.49
SE	540.94	192.37
Minimum	2447.59	16087.10
Maximum	6257.67	6181.95
T-test	1001.90 NS *	
P-value	0.461	
* NS: Non-Significant.		

According to age and sex, Table-5 and Table-6 showed statistical differences in urine MMP-9 levels among age (P<0.05), and significant increase in male group (5078.69 pg/ml) than female (3229.36pg/ml).

Table-5:- Effect of age groups in mean urine MMP-9 level in schistosomal chronic cystitis

Results	Age (years)		
	40-49	50-59	≥60
No. (%)	2 (25.0%)	3 (37.5%)	3 (37.5%)
Mean	2723.65	4937.77	4323.86
SD	390.41	1863.91	1277.66
LSD-value (F.)	1337.52 *		
P-value	0.0391		
* : (P<0.05) Sig			

Table-6:- Effect of gender in MMP-9 level in schistosomal chronic cystitis group

Results	Gender	
	Males	Females
No. (%)	4 (50.0%)	4 (50.00%)
Mean	5078.69	3229.36
SD	1547.76	886.96
T-test	1496.37 *	
P-value	0.00763	
*: Highly Sig.		

Receiver Operating Characteristic (ROC) analysis

ROC was used to determine the accuracy of a any test that classifies subjects into one of two groups, diseased or non, so for diagnosis of cystitis MMP-9 was specific 87.47 % for the diagnosis of the disease and was more sensitive 93.22 % for MMP-9,(Table-7).

Table-7:- The ROC analysis for MMP-9

	Groups	SE	95% CI	Sensitivity (%)	Specificity (%)	P-value
MMP-9	Cystitis	0.02	0.962 -1.00	93.22	87.47	0.001

DISCUSSION

In inflammatory situation, the matrix metalloproteinase-9 has shown to be increased (12). Metalloproteinases are proteolytic enzymes that degrade main components of the basement membrane and extracellular matrix (13).These matrix metalloproteinases associated with the cell surface (14). Also they have a role in angiogenesis specially gelatinase matrix metalloproteinase (MMP-9), (15).

This recent study estimated urine MMP-9 in 50 cystitis patients and in 38 healthy controls, MMP-9 excretion was significantly increased in urine of cystitis patients (3842.90pg/ml) in comparison with the control (634.80pg/ml), (P < 0.0001), This result was in agreement with previous report of (16), as well the active form of MMP-9 measured by ELISA appeared with a significant increase in cystitis patients (17). Normal volunteers had significantly lower level of urinary MMP-9 concentration than groups of patients (18) in response to acute inflammation like cystitis raised in urine level of MMP-9 may be mediated by cytokines elaborated by mast cells and neutrophils. However, no correlation was found between the expression of MMP-9 and inflammation level in cystitis tissues (19).

The levels of urine MMP-9 were significantly different according to age groups, the highest concentration MMP-9 in cystitis patients was in (40-49) age group(4364.38pg/ml) and the lowest was in (60-69) age group (3463.02), and a much higher incidence of cystitis was detected in males compared with females, of whom 36 out of 50 (72.00%) patients were males, 14 (28.00%) were females with (M:F ratio2.6:1), and estimated non-significant (p=0.2774) increase urine MMP-9 level in males than females cystitis patients. The reduce in MMP-9 serum concentrations with advancing in age, as well as, gender with men showing higher number of cystitis patient than women, this is in agreement with the recent epidemiological fact that urinary tract diseases have a higher prevalence in males and in 50> years age group (20).

From present data, out of 50 cystitis patients, only 8 were with schistosomal chronic cystitis which may be due to decrease infection among Iraqi people as a result of treatment. In schistosoma chronic cystitis urine level of MMP-9 (4154.00pg/ml) was increased (but non-significantly)in comparison to that of non-schistosoma chronic cystitis(3783.60pg/ml), there was an agreement with study done by El-Baz *etal.*,(2009), and the level was increased significantly in males 5078.69pg/ml than females 3229.36pg/ml and in (50-59) age group 4937.77pg/ml, during granulomatous pathology due to

schistosomiasis MMP-12 activity regulated the function of tissue-degrading MMP-9 (21). MMP-9 was specific 87.47 % in cystitis patients and was more sensitive 93.22 %, also Hatipoglu *et al.*, (2011) recorded sensitivity 98.6 % and specificity 97.3 %, indicate that MMP-9 works as pro-inflammatory cytokine to regulate immunity and inflammation (11).

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Conclusions

MMP-9 in urine measured by ELISA showed significantly high level in cystitis patients than normal and with non-significant difference in both schistosomal and non schistosomal chronic cystitis.