Iraqi Journal of Cancer and Medical Genetics

Prevalence of matrix metalloproteinase (MMP-9) in urinary bladder cancer

Wasan A. Bakir[1], Noor H. Ismail[1] ,Ekram A. Hassan[2], Ayeda M. Majeed[1],Dina W. Abd[1]

1 Iraqi centre for cancer and medical genetic research / Al- Mustansiryia University
2 Department of pathology/ College of medicine/ Al-Mustansiriya University

Abstract:

Background: Matrix metalloproteinases (MMPs) are a family of extracellular matrix degrading proteinases are involved in tumor invasion, metastasis as well as in the early stages of carcinogenesis,the aim of this study was to explore whether changes in matrix metalloproteinase-9 (MMP-9) are involved in the urinary bladder cancer.
Methods: Expression of MMP-9 was evaluated in the tumor of 30 urinary bladder cancer cases and 20 control group with in situ hybridization.
Results: Increased expression of MMP-9 was significantly higher in tumor compared with normal samples. Expression of MMP-9 did not found any significant association with tumor age, sex, grade and muscle invasion.
Conclusion: This study shows that MMP-9 may play an essential role in the determination of bladder cancer.

Keywords: *MMP-9, Bladder carcinoma*

Introduction:

Bladder cancer when superficial has a good prognosis but it has a high recurrence risk and about 10–15% of the superficial carcinomas will progress into muscle invasive or metastatic type (1). The most powerful factor for predicting the behavior of bladder carcinoma is the stage of the tumor. One of the essential alterations that occur in malignancy is tissue invasion and metastasis (2). The metastatic process involves intravasation and extravasation of tumor cells, followed by reimplantation of tumor cells, formation of a new tumor stroma, and neoangiogenesis to consolidate a secondary tumor at a distant site (3). Degradationof the extracellular matrix and components of the basement membrane by proteases facilitates the detachment of tumor cells, their crossing of tissue boundaries, and invasion into adjacent tissue compartments (2).

The importance of tumor-associated proteases in invasion and metastasis has been demonstrated for a variety of solid malignant tumors (4).

Corresponding Address:
Noor Hashim Ismail
Iraqi center for cancer and medical genetic research Al-Mustansiriyah University
Email: nooraldhahir@yahoo.com

Matrix metalloproteases (MMPs) comprise a family of proteolytic enzymes that have been implicated in tumor growth, invasion and metastasis in experimental cancer models and inhuman tumors (5). Two members of this family in particular, MMP-2 and MMP-9, degrade typeIV collagen, fibronectin and laminin, major components of the basement membrane and are commonly used as markers of the malignant phenotype. MMP activity is regulated by a group of four distinct tissue inhibitors of metalloproteases (TIMPs) (6).
Matrix metalloproteases are characterized by a zinc atom at the active site and are classified according to homologies in sequence and substrate affinity (7).One of the first observations that suggested a role for MMP-9 in tumor invasion relates to the fact that the release of MMP-9 is associated with the metastatic phenotype of transformed rat embryo cells (8).

Matrix metalloproteases-9 (Gelatinase B, 92kDa type IV collagenase) was first purified from human macrophages (9). MMP-9 expression is limited to osteoclasts, macrophages, trophoblasts, hippocampal neurocytes and migrating keratinocytes and it is controlled by growth factors, chemokines and other stimulatory signals (10). MMP-9 is secreted as an inactive precursor form, proMMP-9. It forms a tight complex with TIMP-1 and TIMP-3. The complex of proMMP-9 and TIMP-1 is a potential inhibitor of MMPs (11). Another activation mechanism is plasminogen/MMP-3 mediated activa-

tion. Plasmin can also directly activate proMMP-9 (10).
The role of MMP-9 is, however, not that clear particularly in bladder cancer (12). The results have also been conflicting in other types of cancer and the expression of MMP-9 has been linked to both favorable and poor prognosis (13).

The aim to explore whether MMP-9 is involved in urinary bladder cancer and to investigate the association between tumor MMP-9 and theclinicopathological features of bladder cancer and MMP-9 as a prognosticmarker in bladder carcinoma.

Material and Methods:

Thirty urinary bladder carcinoma with paraffin embedded tissue samples were obtained from the files of the Department of Pathologyat Al-Yarmouk and Baghdad Teaching Hospital. In addition 20 apparently normal bladder autopsies were collected from the Forensic Medicine Institute archives. The samples were evaluated by a pathologist to represent the carcinomaof the bladder. The histological grade of the tumors was reviewed and classified according to the WHO Classification for urological tumors. The median age of the patients was 59 years(49-82). The clinical stage was defined according to the 1997 International Union against Cancer tumor staging system.

In situ hybridization (ISH) for detection of MMP-9 gene.

The use of Biotin – Labeled DNA probe for mmp-9 / DNA (Maxim Biotic, USA) 216bp, MMP-9 (8 □g/100 µl) litter dd H2O (Maxim Biotech, Inc., U.S.A).

In situ hybridization (ISH) is a technique makes use of the high specificity of complementary nucleic acid binding to detect specific DNA or RNA sequence in the cell. For detection of this markers , the biotinylated DNA probe hybridize to the target sequence (mmp-9 mRNA sequence) then a streptavidin-AP (streptavidin-alkaline phosphatase) Conjugate is appliedfollowed by addition of the substrate promo-chloro – indolyl – phosphatel / nitro-blue tetrazolium (BCIP/NBT) which yield an intense blue – black signal appears at the directly specific site of the hybridized probe. This streptei-vidin – Ap conjugate like the biotinylated probe provides a rapid and highly sensitive detection method.Hybridization / Detection System will give an intense blue –black color at the specific sites of the hybridization probe in both positive test tissues. Evaluation of the in situ staining was done with assistance of a histopathologist.

Scoring

A scoring system that includes evaluation of the staining percentage of stained bladder cells was employed for the expression of MMP-9 .Counting the number of the positive cells in the bladder tissue which gave a blue-black nuclear staining under the light microscope. The extent of the ISH signaling the cells of the examined tissue was determined in 10 fields under high power microscope (100X). In each field, the total staining score divided by the number of whole cell per field in 10 fields, so the percentage of positively stained cells in the 10 fields was calculated for each case by taking the mean of the percentage of the positively stained cell in the 10 fields. Tissues were regarded MMP-9 positive when their ISH signaling scores were ≥ 5% (10).

Statistical analysis: The associations between the presences of MMP - 9 in different groups were assessed by the Chi-square test. P value of <0.05was considered statistically significant.

Result:

As shown in table -1 overexpression of MMP-9 gene was detected in 70.57% (26 outof 30) of the urinary bladder cancer samples (≥50% of the cells appearing aspositive). The remaining 4 samples were showed a weakexpression-for MMP-9and there were a highly significant difference ($p<0.01$) in the mean percentage of MMP-9 mRNA expression in tissue of bladder carcinoma and bladder normal tissue. The expression of MMP-9 was heterogeneous dark brown staining in the tissue shown in figure - 1

Table 1: Scoring of MMP – 9 expressions in normal control and patients with bladder carcinoma.

Groups	No	< 5%	> 5% - <25	>25% - <50%	> 50%	p-value
Bladder carcinoma	30	0	2(20.7%)	2(42.7%)	26(70.57%)	*p<0.01
Control	10	13(70.57%)	6(12. 05%)	1(26%)	0	

* Significant at the < 0.01 level.

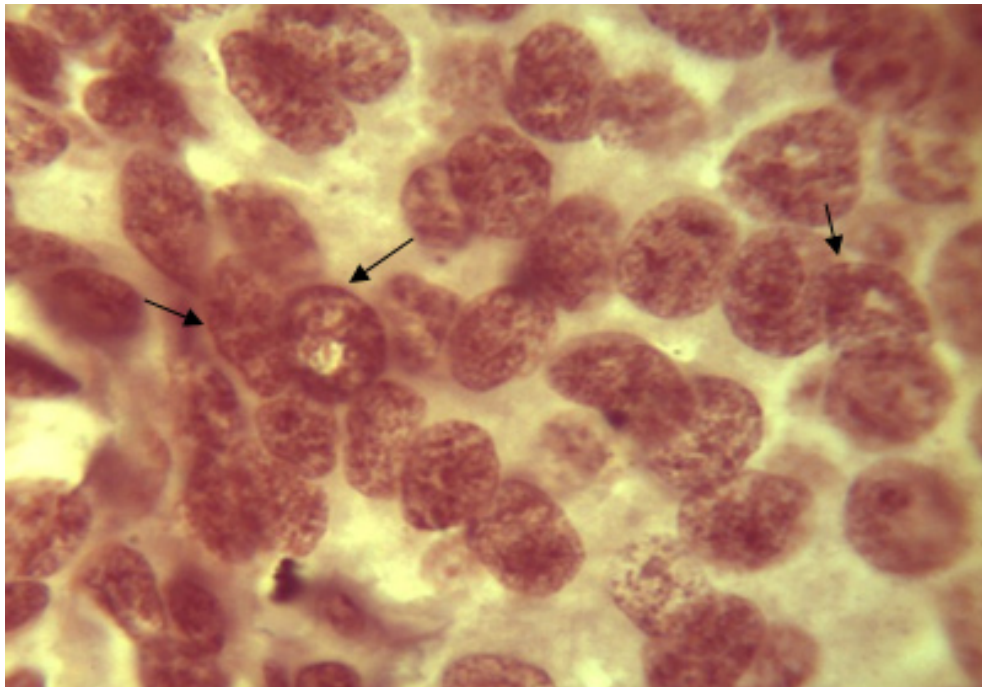

Figure 1: Detection of MMP–9 mRNA, in patients with bladder carcinoma by in situ hybridization. Staining of MMP – 9 by BCIP/ NBT - Chromogen (blue-black) counter-stained with nuclear fast red. Tissue from patients with bladder carcinoma shows positive MMP-9 by hybridization signals.

A pooled analysis of the positivity of the different of expression in tissue is presented in Table 2. There was no statisticalsignificant between MMP-9 positivity and sex or age (P>0.05).

Also, there was nosignificant difference between the overexpression of MMP-9 and tumor grade orwith muscle invasion (P>0.05).

Table 2: Clinical and pathological feature of 30 patients with bladder carcinoma.

Variable	MMP-٩ in situ hybridization				*p*-value
	<5%	>5%-<25%	>25% - <50%	> 50%	
Sex					
Male	0	2(20.5%)	1(49.4%)	15(70.42%)	Ns.
Female		0	1(36%)	11(70.78%)	(P>0.05)
Age (years)					
≤60 y	0	0	3(36%)	14(69.86%)	Ns.
>60 y		2(20.5%)	1(49.4%)	10(71.7%)	(P>0.05)
Tumor grade					
Low grade	0	2(20.5%)	2 (42.7%)	15(67.8%)	Ns.
High grade		0	0	11(74.34%)	(P>0.05)
Muscle invasion					
With Muscle invasion	0	1(17)	0	14(72.19)	Ns.
Without Muscle invasion		1(24)	2(42.7)	12(68.68)	(P>0.05)

Ns. Non significant

Discussion:

Bladder carcinoma is one of the most common urologic malignancies occurring worldwide (14, 15). Matrix metalloproteinase - 9 (MMP-9) has been associated with tumor cell invasion and metastasis in many human cancers, including bladder cancer. As a marker for elevated urinary mRNA levels of MMP-9 are associated with cancer (16). High MMP-9 levels were significantly correlated with large tumor size and poor malignancy grade, and increasing levels were associated with poor overall survival (17).

Our results revealed high frequency of MMP-9 activities in bladder cancer cases as compared to control ($p<0.001$). 70% of the urinary bladder carcinomas, showed an overexpression (>50%) of MMP-9 mRNA. MMP-9 degrades type IV collagen, the major component of the basement membrane (18). Studies that measured urinary MMP-9 by ISH have suggested that MMP-9 levels demonstrated that urinary MMP-9 levels were significantly elevated in bladder cancer subjects by ISH analysis (19).Several independent studies as well as our present study have consistently reported elevated MMP-9 activity in urine samples from patients with bladder cancer which provided the rationale to test MMP-9 as a screening of diagnostic marker(20).

Here, MMP-9 positivity did not correlate to the age, sex, grade and muscle invasionof the tumor not observed. In other studies by different investigators, correlation with clinicopathological parameters varies (21, 22). The results were consisted with of the study who did not found any relation between the expression of mmp-9 in bladder carcinoma tissue with clinicopathological feature (23).

One explanation for the discovery in the present study of MMP-9 may be the possible role of MMP-9 in immunological host reactions in cancer patients or incontrolling tumor angiogenesis. It is possible that these factors might dominate in early tumor progression (24). MMP-9, similarly to other MMPs, is produced asan inactive, latent form that requires activation for being enzymatically active Stromelysin-1 (MMP-3) is one of the most effective activators for MMP- 9 (25).

Earlier studies showed the potential importance of these enzymes in tissue culture and animal models of invasion and metastasis (26, 27). MMP-9 similarly to other MMPs is produced as an inactive, latent form that requires activation for being enzymatically active (27).

MMP-9, may participate in the inhibition of endothelial cell proliferation and angiogenesis. This is due to the fact that MMPs, including MMP-9, generate antiangiogenicendostatin-containing peptides from collagen XVIII (28(. In the present study, we did not find a statistical correlation between MMP-9 staining and tumor grade or stage. This is in line with Durkan et al. who also did not found any correlation between MMP-9 staining and histological grade and stage (28).

In addition other MMPs such as MMP-2, MMP-7 and MMP-13 and some serine proteinases are able to activate proMMP-9 (29). MMPs are known to be needed in degradation of extracellular matrix and basement membranes, but they also play a role in the formation of new capillaries, which is essential for tumor progression.MMP-9 is transcriptionally regulated by inflammatory cytokines such as tumor necrosis factor and interleukins (30).

Conclusion:

We have found that MMPs overexpression in urinary bladder carcinomais associated withbetter prognosis and might have adiagnostic value but a further studies are needed to confirm this finding.

References:

1. Roy, R.; Louis, G.; Loughlin, K.; , Wiederschain, D.; Kilroy, S.; Lamb, C.; Zurakowski, D. and Moses. M. (2008):Tumor-Specific Urinary MMP Fingerprinting: Identification of High Molecular Weight Urinary MMP Species.Clin Cancer Res.;14: 6610–6617.
2. Roy, R.; Zhang, B. and Moses, M. (2006): Making the cut: protease-mediated regulation of angiogenesis. Exp Cell Res 312:608–622.
3. Hegarty, P. (2012):MMP-2 and MMP-9 in lymph-node-positive bladdercancerJ ClinPathol.;65:470-471.
4. Schmalfeldt, B.; Prechtel, D.;Ha¨rting, K.;Spa¨the, K.; Rutke, S.; Konik, E.; Fridman, R. and Berger, U. (2001):Increased Expression of Matrix Metalloproteinases (MMP)-2, MMP- 9, and the Urokinase-Type Plasminogen Activator Is Associated with Progression from Benign to Advanced Ovarian Cancer.Cancer Res;7:2396-2404.
5. Bergers, G.; Brekken, R. and McMahon, G. (2000): Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol.; 2:737–744.
6. Roy,R.; Louis,G.; Loughlin,K.;Wiederschain,D.; Kilroy,S.; Lamb,C.; Zurakowski,D. and Moses, M. (2008):Tumor-Specific Urinary MMP Fingerprinting: Identification of High Molecular Weight Urinary MMP Species; Clin Cancer Res.14: 6610–6617.
7. Rodriguez Faba, O.; Palou-Redorta, J.; Fern´andez-G´omez, J.; Algaba, F.; Eir ´ o, N.; Villavicencio,1 H. and Vizoso, F. (2012):MatrixMetalloproteinases and Bladder Cancer:What is New? ISRN Urology.Article ID 581539, 9 pagesz.
8. Feng,N.; Rom, W.; Beland, F. and Tang, M. (2002): 4Aminobiphenyl is a major etiological agent of human bladder cancer: evidence from its DNA binding spectrum in humanp53 gene.Carcinogenesis; 23:1721–1727.
9. Vartio, T.; Hovi,T. and Vaheri, A. (2003): Human macrophages synthesize and secrete a major 95,000-dalton gelatin binding protein distinct from fibronectin. J Bi-

olChem257: 8862–8866.
10. Vasala, K.;Pääkkö, P. andTurpeenniemi-Hujanen, T. (2008): Matrix metalloproteinase 9 (MMP-9) immunoreactiveproteins in urinary bladder cancer: A marker of favorableprognosis. Anticancer Res 28: 1757–1761.
11. Visse, R.& Nagase, H. (2003): Matrix metalloproteinases and tissue inhibitors ofmetalloproteinases: structure, function and biochemistry. Circ Res.; 92: 827–839.
12. Durkan, G.; Nutt, J.; Marsh, C.; Rajjayabun, P.; Robinsin, M.; Neal, D.; Lunec, J. and Mellon, K. (2003): Alteration in urinary matrix metalloproteinase-9 to tissue inhibitor of metalloproteinase-1 ratio predicts recurrence in non-muscle, invasive bladder cancer. Clin Cancer Res9: 2576-2582.
13. Scorilas, A.; Karamelis, A.; Arnogiannaki, N.; Ardavanis, A.; Bassilopulos, P.; Trangas, T. and Talieri, M. (2001): Overexpression of matrix-metalloproteinase-9 in human breast cancer: a potential favorable indicator in node negative patients. Br J Cancer.;84: 1488-1496.
14. Nutt, J.; Durkan, G.;Mellon, J.andLunec, J. (2003): Matrix metalloproteinases (MMPs) in bladder cancer: the induction of MMP9 by epidermal growth factor and its detection in urine.BJU Int.; 9:99-104.
15. Jemal, A., T. Murray, E. Ward, A Samuels, R.C. Tiwari, A. Ghafoor, E.J. Feuer and M.J. Thun, 2005. "Cancer Statistics, 2005." CA A Cancer Journal for Clinicians 55 (1): 10-30.
16. Habuchi, T.; Marberger, M, and Droller, M. (2005): Prognostic markers for bladder cancer: International Consensus Panel on bladder tumor markers. Urology 2005, 66:64.
17. Offersen, B.; Knap, M. andHorsman, M. (2010): Matrix metalloproteinase-9 measured in urine from bladder cancer patients is an independent prognostic marker of poor survival. ActaOncol.;49:1283.
18. Fouda, M.; Mohammed, M.; Sharada, H.;Abdalla, M. and Abdel Wahab, A. (2012):Determination of MMP-2 and MMP-9 activities in urine of schistosomal bladder carcinoma: A diagnostic tumor biomarkersJournal of Applied Sciences Research, 8(6): 3097-3102, 2012.
19. IL-8 as a urinary biomarker for the detection of bladder cancer Virginia Urquidi1, Myron Chang2, Yunfeng Dai2, Jeongsoon Kim1, Edward D Wolfson3, Steve Goodison1 and Charles J RosserUrquidi et al. BMC Urology 2012, 12:12.
20. Tibor, S.;Dorp, F;Ergün, S. and Rübben, H. (2011): Matrix metalloproteinases and their clinical relevance in urinary bladder cancer.Nature reviews Urology, 8: 241-254
21. Durkan GC, Nutt JE, Marsh C, Rajjayabun PH, Robinson MC, Neal DE, Lunec J & Mellon K (2003) Alteration in urinary matrix metalloproteinase-9 to tissue inhibitor of metalloproteinase-1 ratio predicts recurrence in nonmuscle-invasive bladder cancer. Clin. Cancer Res.;9: 2576–2582.
22. Özemir E, Kakehi Y, Okuno H & Yoshida O (1999) Role of matrix metalloproteinase-9 in the basement membrane destruction of superficial urothelial carcinomas. J Urol.; 161: 1359–1363.
23. Ozdemir, E.; Kakehi, Y.; Okuno, H. and Yoshida, O. (1999): Role of matrix metalloproteinases – 9 in the basement membrane destruction of superficial urothelial carcinomas. J Urol.; 161: 1359 – 1363.
24. Talvensaari-Mattila A &Turpeenniemi-Hujanen (2005) preoperative serum MMP-9 immunoreactive protein is a prognostic indicator for relapse-free survival in breast carcinoma. Cancer Let.; 20: 237–242.
25. Sternlicht MD &Werb Z (2001): How matrix metalloproteinases regulate cell behavior. Ann Rev Cell Biol.; 17: 463–516.
26. Ruokolainen H, Pikk P and Turpeenniemi-Hujanen T: Expression of matrix metalloproteinase-9 in head and neck squamous cell carcinoma: A potential marker for prognosis. Clin Cancer Res 10: 3110-3116, 2004.
27. Rahko E, Jukkola A, Melkko J, Pکkk؟n P, Bloigu R Talvensaari- Mattila A and Turpeenniemi-Hujanen T: Matrix metalloproteinase- 9 (MMP-9) immunoreactiveprotein has a modest prognostic value in locally advanced breast carcinoma patients treated with an adjuvant antiestrogen therapy. Anticancer Res 24: 4247-4253, 2004.
28. Szarvas, Tibor, Frank VomDorp, SüleymanErgün, and Herbert Rübben, 2011. "Matrix metalloproteinases and their clinical relevance in urinary bladder cancer." Nature reviews Urology, 8(5): 241-254.
29. Eissa, S.;Labib, A.; Bassiony, M.; Tash, F. andEl-Zayat, T. (2007):Noninvasive diagnosis of bladder cancer by detection of matrix metalloproteinases (MMP-2 and MMP-9) SwellamMand their inhibitor (TIMP-2) in urine.European Urology ; 52:1388-1396.
30. Fouda, M.; Mohammed, M.; Sharada, H.; Abdalla, M. and Abdel Wahab, A. (2012): Determination of MMP-2 and MMP-9 activities in urine of Schistosomal bladder carcinoma: A diagnostic tumor biomarkers. Journal of Applied Sciences Research, 8: 3097-3102.

انتشار 9 - Matrix metalloproteinases - (MMP-9) في سرطان المثانةالبولية

وسن عبدالاله باقر[1]، نور هاشم اسماعيل[1]، اكرام عبد اللطيف حسن[2]، عايدة ممدوح[1]، دينا وائل[1]
1 المركز العراقي لبحوث السرطان والوراثة الطبية / الجامعة المستنصرية
2 كلية الطب /فرع الامراض / الجامعة المستنصرية

الخلاصة:

Matrix metalloproteinases هي عائلة من الانزيمات الخارج خلوية المحطمة للبروتينات التي تشارك فيغزو الورم، وكذلك ورم خبيث في المراحل المبكرة من السرطانوالهدف من هذه الدراسة:هو استكشاف ما إذا كان MMP-9 يشارك في تطور سرطان المثانة البولية.
المواد وطرق العمل: تم تقييم التعبير عن MMP-9 في ورم 30 حالات سرطان المثانة البولية و20 مجموعة سيطرةبطريقة التهجين في الموقع: الأساليب.
النتائج: كان التعبير زيادة MMP-9 أعلى بكثير في الورم مقارنة مع العينات الطبيعية. لم يتم العثور على أي ارتباط معنويبينMMP-9 مع تقدم العمر والجنس والدرجة وغزو العضلات
الاستنتاج: هذه الدراسة تبين أن تعبيرMMP-9 يلعب دور مهم في سرطان المثانة .