Immunohistochemical expression of matrix metalloproteinase-9 in urothelial carcinoma of urinary bladder

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Context
Bladder urothelial carcinoma is one of the most common cancers in both men and women worldwide. Matrix metalloproteinases (MMPs) are a family of endopeptidases capable of degrading essentially all extracellular matrix components. MMP-9 has multiple substrates; however, collagen type IV is the most crucial substrate. The proteolytic activity of MMP-9 not only promotes invasion and metastasis but also releases matrix-bound growth factors and other signaling molecules, participating in growth, angiogenesis, and inflammatory responses.
Aims
To evaluate the expression of MMP-9 in urothelial carcinoma of the bladder immunohistochemically, to correlate the expression with stromal invasion, tumor grade, and histological type, and to test for the usefulness of MMP-9 as ancillary test to distinguish different histological types and tumor grades
Settings and design
A retrospective study included the collection of 37 formalin-fixed, paraffin-embedded tissue blocks. Overall, 32 blocks/cases represent urothelial carcinoma of urinary bladder and five blocks/cases of reactive (inflammatory) conditions.
Patients and methods
Two sections from each case were stained with hematoxylin and eosin and immunohistochemically with MMP-9 antibodies, using rabbit monoclonal anti-human MMP-9 as a primary antibody.
Statistical analysis
It was performed with statistical package for the social sciences (SPSS Inc., Version 18.0, Chicago) and Excel 2007 program. Data analysis was done using *t* test and χ^2 test.
Results
Eighteen (56.25%) cases of the 32 total cases of urothelial carcinoma included in this study show a negative immunohistochemical expression for MMP-9 and 14 (43.75%) cases were positive for MMP-9. There was no statistical significant correlation between MMP-9 expression and tumor stage, grade, and histological type.
Conclusion
No statistical significance was found between MMP-9 immunohistochemical expression and the studied prognostic parameters: histological type, grade, and stage.

Keywords:
matrix metalloproteinase 9, urinary bladder, urothelial carcinoma

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Introduction

Worldwide, bladder cancer is the seventh most common cancer in men and the 17th most common cancer in women [1]. In the developed world, it ranks as the fourth and ninth most common cancer in men and women, respectively [2].

Urothelial carcinoma (traditionally known as transitional cell carcinoma) is the most frequently occurring type of bladder cancer and accounting for ~90% of all primary tumors in the urinary bladder [3,4].

Matrix metalloproteinases (MMPs) are a family of more than 24 human zinc-dependent endopeptidases collectively capable of degrading essentially all extracellular matrix (ECM) components, so they have a role in tumor growth, invasion, and metastasis. According to their structure and substrate specificity, members of MMPs family can be classified into subgroups of collagenases, stromelysins, gelatinases, membrane-type MMPs, and other MMPs [4,5].

MMP-9 has multiple substrates; however, collagen type IV, the main component of basement membranes, is the most crucial MMP-9 substrate in

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a tumor microenvironment [6]. The proteolytic activity of MMP-9 against collagen type IV not only promotes invasion and metastasis but also releases matrix-bound growth factors and other signaling molecules to promote growth signaling, angiogenesis, and an inflammatory response [7,8].

The ECM is the first barrier that cancer cells must cross to metastasize. First, they pass through the epithelial basement membrane; after that, the cancer cells must invade into the surrounding stroma. Thereafter, they enter blood vessels or lymphatics and extravasate to distant organs to make new proliferating tumors. This process is composed of degradation of ECM and cell adhesion but also angiogenesis in the early stages of tumor development [9].

MMPs are thought to facilitate invasion and metastasis by degradation of ECM components. MMPs also mediate the activation of growth factors, suppression of tumor cell apoptosis, and destruction of chemokine gradient development by host immune response or the release of angiogenic factors [9,10]. MMPs can also target substrates and influence the apoptotic process, which is also important for tumor prognosis [9].

In general, MMPs contain a signal peptide, a propeptide, a catalytic domain with the highly conserved zinc-binding site, and a hemopexin-like domain linked to the catalytic domain by a hinge region. In addition, gelatinase-A (MMP-2) and gelatinase-B (MMP-9) contain fibronectin type II inserts within the catalytic domain, and membrane type (MT-MMPs) contain a transmembrane domain in the carboxyl-terminal end of the hemopexin-like domain. The hemopexin domain is absent in the smallest MMP, matrilysin (MMP-7) [11].

Expression of most MMPs is normally low in tissues and is induced when remodeling of ECM is required. MMP gene expression is primarily regulated at the transcriptional level, but there is also evidence about modulation of mRNA stability in response to growth factors and cytokines [12,13].

All MMPs are synthesized in the latent form (zymogen). They are secreted as proenzymes and require extracellular activation. They can be activated *in vitro* by many mechanisms including organomercurials and other proteases [14].

The MMPs are inhibited by specific endogenous tissue inhibitor of metalloproteinases (TIMPs), which comprise a family of four protease inhibitors: TIMP-1, TIMP-2, TIMP-3, and TIMP-4 [15].

Previous studies have revealed that MMP-9 is involved in the pathogenesis of bladder cancer [7,16–18]; however, alternative studies have failed to establish an association between MMP-9 expression and bladder cancer, leading to the conclusion that MMP-9 may not be an effective marker for bladder cancer detection [19,20].

This study was conducted based on the aforementioned importance of MMPs in general and MMP-9 in particular in various physiological and pathological processes, and in view of the conflict in the available data regarding its presumed role in the pathogenesis of urinary bladder urothelial carcinoma, it aimed to evaluate the expression of MMP-9 in urothelial carcinoma of the bladder immunohistochemically and to correlate the expression with stromal invasion, tumor grade, and histological type and to test for the usefulness of MMP-9 as ancillary test to distinguish different histological types and tumor grades

Patients and methods

Formalin-fixed, paraffin-embedded tissue blocks were collected from the archived materials of Ghazi Al Hariri specialized surgical hospital in Baghdad covering the period from February to July 2017.

The paraffin blocks represent 32 cases of bladder urothelial carcinoma, where five cases were cystectomy specimens and 27 cases were transurethral biopsy specimens, in addition to five cases of reactive (inflammatory cystitis) conditions.

Clinicopathological parameters such as pathological stage and histological grade of the tumors were obtained from the available histopathological reports.

Two sections of 5-μm thickness were taken from each block: the first was stained with hematoxylin and eosin stain for histological revision (reassessed for histological tumor type, grade, and invasiveness), and the other section was stained immunohistochemically for MMP-9, using rabbit monoclonal anti-human MMP-9 (PathnSitu, CA, USA) as a primary antibody and polyExcel HRP/DAB Detection system (PathnSitu USA) as a secondary detection system with hematoxylin counter stain. A microwave oven was used for antigen retrieval.

A technical negative control was done by omitting the primary antibody, and colorectal adenocarcinoma

sections were used as a positive control (as recommended by the antibody manufacturer's leaflet).

The percentage of positively stained tumor cells with cytoplasmic staining was used to evaluate and classify results as following:

(1) Less than 5% was negative.
(2) More than 5–25% indicate low level (+).
(3) More than 25% but less than 50% is intermediate staining (++).
(4) More than 50% indicate high level of staining (+++).
(5) Only cases with moderate or marked expression patterns (scores 2 or 3) were considered positive [9,21].

Statistical analysis

Statistical analysis was performed with statistical package for the social sciences (version 18.88) and also Excel 2007 programs. Data analysis was done using t test, χ^2 test for tables with frequencies, percentages, ranges, means SD, and standard errors of mean. Values were considered statistically significant when P value less than 0.05.

Results

Distribution of cases according to the studied parameters

Histological type of carcinoma

Eighteen (56.25%) cases were papillary high-grade urothelial carcinoma, whereas seven (21.88%) cases were papillary low-grade urothelial carcinoma, with the same percentage for invasive solid type.

Tumor grade

Twenty-five (78.13%) cases were high-grade tumors, whereas the rest of the cases (seven cases; 21.88%) were low-grade urothelial carcinomas.

Tumor stage: T parameter

Twenty-five (78.13%) cases were in stage T1 (according to AJCC 7th edition staging system), whereas five (15.63%) cases were in stage T2, and one (3.125%) case was in stage Ta and T3.

Correlation of matrix metalloproteinase-9 expression and the various studied parameters

Overall, 18 (56.25%) cases showed a negative immunohistochemical expression for MMP-9 and 14 (43.75%) cases were positive for MMP-9.

All the reactive/inflammatory cystitis cases were immunohistochemically negative for MMP-9 (Fig. 1).

Matrix metalloproteinase-9expression according to histological type of carcinoma

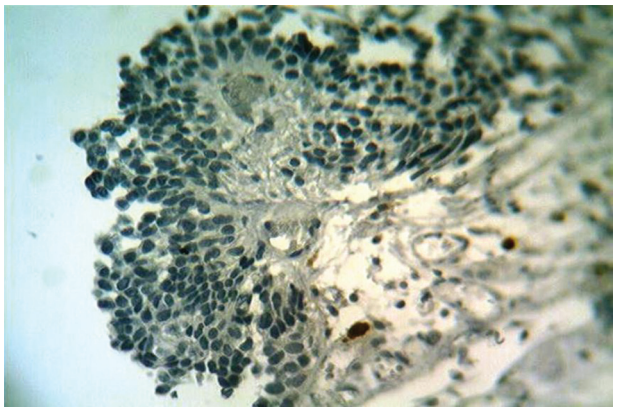
A positive immunohistochemical expression for MMP-9 was found in seven (38.9%) cases of papillary high-grade urothelial carcinomas (Figs 2 and 3) and was negative in the rest of the cases (11 cases; 61.1%).

For low-grade papillary urothelial carcinomas, four (57.1%) cases were positive (Fig. 4) and three (42.9%) cases were negative for MMP-9.

Three (42.9%) cases of solid invasive variant show a positive immunohistochemical expression for MMP-9 (Fig. 5) and four (57.1%) cases were negative for the marker.

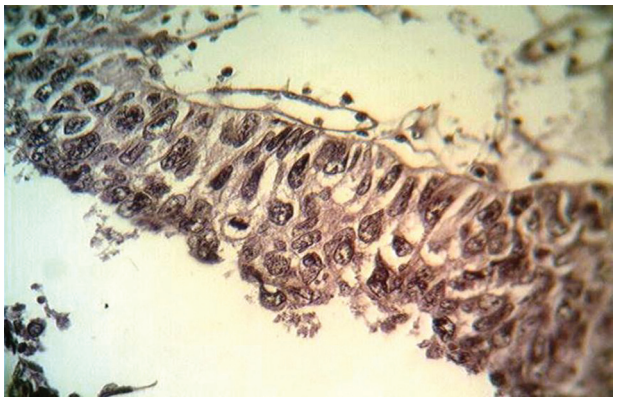
There was no significant statistical correlation between the histological type of the tumor and the immunohistochemical expression of MMP-9, with P value of 0.685 as shown in the Table 1.

Figure 1



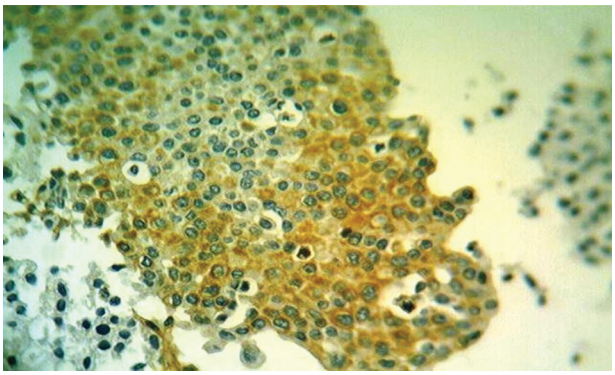
Reactive urothelial hyperplasia in chronic nonspecific cystitis showing negative immunohistochemical for MMP-9 (×40). MMP, matrix metalloproteinase.

Figure 2



High-grade papillary urothelial carcinoma (hematoxylin and eosin, ×40).

Figure 3



High-grade papillary urothelial carcinoma invading the underlying stroma (T1) showing positive cytoplasmic expression of MMP-9 by the malignant urothelial cells (×40). MMP, matrix metalloproteinase.

Matrix metalloproteinase-9 expression according to tumor stage

Only one of the studied cases was Ta stage and was positive for MMP-9 immunohistochemical expression. Eleven (44%) cases of T1 stage were positive for MMP-9 expression, whereas 14 (56%) cases were negative.

Two cases of T2 stage showed positive expression of MMP-9 whereas the rest three (60%) cases were negative for the marker. Only one of the studied cases was T3 stage, and it shows a negative immunohistochemical expression for MMP-9.

There was no significant statistical correlation between MMP-9 expression and tumor stage, with *P* value of 2.093, as shown in Table 2.

Matrix metalloproteinase-9 expression according to tumor histological grade

Finally, regarding tumor grade, 10 (40%) cases of high-grade tumors showed positive immunohistochemical expression of MMP-9 (Figs 2 and 3) and the rest 15 (60%) cases were negative for MMP-9 (Fig. 6).

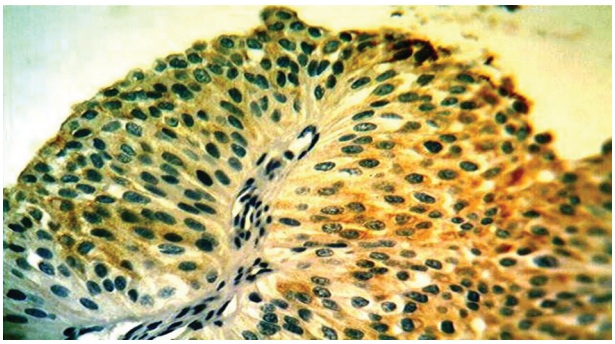
Four (57.1%) cases of low-grade tumors showed positive immunohistochemical staining for MMP-9 (Fig. 4) and three (42.9%) cases were negative for the marker.

There was no significant statistical correlation between MMP-9 expression and tumor grade, with *P* value of 0.653, as shown in Table 3.

Discussion

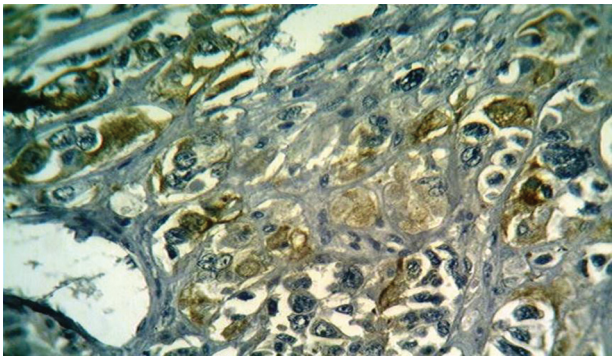
Urinary bladder cancer is a common cancer globally, with an estimated 430 000 new cases diagnosed in 2012 [22].

Figure 4



Low-grade papillary urothelial carcinoma, noninvasive, showing positive cytoplasmic expression of MMP-9 by the malignant cells (×40). MMP, matrix metalloproteinase.

Figure 5



High-grade urothelial carcinoma, solid type, invading the underlying stroma (T1) showing positive cytoplasmic expression of MMP-9 by the malignant urothelial cells (×40). MMP, matrix metalloproteinase.

Table 1 Correlation of matrix metalloproteinase-9 expression and histological tumor type

Type	MMP-9 [*N* (%)]		*P* value
	Positive	Negative	
Papillary high grade	7 (38.9)	11 (61.1)	0.685
Papillary low grade	4 (57.1)	3 (42.9)	
Invasive solid	3 (42.9)	4 (57.1)	

MMP, matrix metalloproteinases.

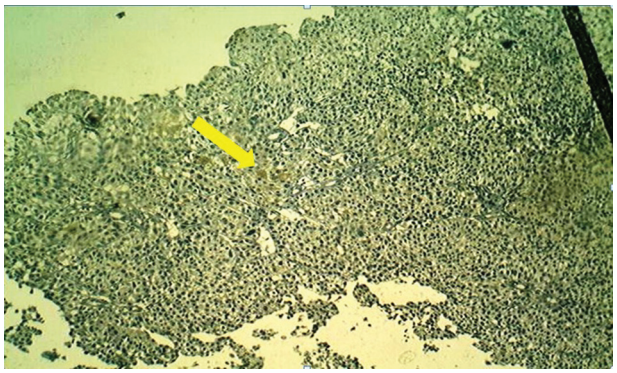
Table 2 Correlation of matrix metalloproteinase-9 expression and tumor stage

Stages	MMP-9 [*N* (%)]		*P* value
	Positive	Negative	
Ta	1 (100)	0 (0)	2.093
T1	11 (44)	14 (56)	
T2	2 (40)	3 (60)	
T3	0 (0)	1 (100)	

MMP, matrix metalloproteinase.

Various risk factors have been shown to be implicated in the etiology and pathogenesis of this tumor including smoking tobacco, chronic inflammatory

Figure 6



High-grade papillary urothelial carcinoma, T1, showing focal cytoplasmic expression of MMP-9 (arrow) by the malignant urothelial cells (considered as negative) (×10). MMP, matrix metalloproteinase.

Table 3 Correlation of matrix metalloproteinase-9 expression and tumor grade

Grades	MMP-9 [*N* (%)]		*P* value
	Positive	Negative	
Low grade	4 (57.1)	3 (42.9)	0.653
High grade	10 (40.0)	15 (60.0)	

MMP, matrix metalloproteinases.

states involving the bladder, and exposure to radiation and chemical carcinogens like aromatic amines and chemotherapeutic agents [4].

The present study investigated the immunohistochemical expression status of one of the 25 members of the MMP family, namely MMP-9, in urinary bladder urothelial carcinoma.

Since 1962 when the first MMP ever discovered in a tadpole [23], this family of proteinases has emerged as a very important player in normal physiological and pathological processes including neoplasia.

Since then, a significant number of studies have been conducted to clarify their role and to test the effectiveness of their inhibitors.

MMP9, also called gelatinase B and one of the soluble MMPs members, has a wide range of functions in normal physiologic processes stemming from its ability to degrade ECM, being a key regulator of skeletal remodeling [24], neutrophil crossing of basement membrane during inflammatory reactions [25], angiogenesis regulator [26], among many others.

However, the presumed role of MMP-9 in various aspects of tumor biology was and still the focus of extensive research, and the relation between this protease and cancers is a complex one that still demands further study.

In the present study, 32 cases of bladder urothelial carcinoma were examined for the expression of MMP-9 protein in tumor cells, taking into consideration the fact that MMP-9 is predominantly secreted by neoplastic cells unlike many other members of this protease family.

Most cases (18/32 cases) (56.25%) were papillary high-grade urothelial carcinoma, whereas seven (21.88%) of 32 cases were papillary low-grade urothelial carcinoma and another seven (21.88%) of the 32 cases were of the invasive solid type.

The results showed that there was no significant statistical correlation between the histological type of the tumor and the immunohistochemical expression of MMP-9, with *P* value of 0.685.

Most of cases (25/32) (78.13%) were in stage T1 (according to AJCC 7th edition staging system), whereas five (15.63%) cases were in stage T2, and one (3.125%) case was in stage Ta and T3.

There was no significant correlation between MMP-9 expression and tumor stage, with *P* value of 2.093. This result was compatible with Yassen and colleagues who detected a nonsignificant correlation between MMP-9 expression and tumor stage, with *P* value of 0.286[27], and that of Vasala [9], with the same nonsignificant correlation and a *P* value of 0.78.Regarding tumor grade, most of our cases (25/32 cases) (78.13%) were high-grade tumors, whereas the rest of the cases (7/32) (21.88%) were low-grade urothelial carcinomas.

There was no significant statistical correlation between MMP-9 expression and tumor grade (*P*=0.653). Moreover, 40% of high-grade tumors showed positive immunohistochemical staining with MMP-9 and 60% were negative, whereas 57.1% of low-grade tumors were positive for MMP-9 and 42.9% were negative. Yassen *et al*. [27] found a nonsignificant correlation between tumor grade and MMP-9 expression, with a *P* value of 0.213. Vasala [9] detected a nonsignificant correlation between MMP-9 expression and the grade of urothelial carcinoma, with a *P* value of 0.25.

On the contrary, Zeng *et al*. [4] detected a significant correlation between MMP-9 expression and tumor grade (more expression with high-grade tumors than low-grade ones) and had reported that the expression

rates of MMP-9 between G1/G2 and G3 tumors demonstrated significant differences in Asian ($P<0.001$) and white patients ($P=0.011$), whereas there was no clear difference in African patients ($P=0.632$). This disagrees with the results of the present study, which may be owing to the difference in sample size and in the grading system (three tiered instead of two tiered system).

Conclusion

(1) MMP-9 was positively expressed in 43.75% of the studied cases of urothelial carcinoma and was negative in all the reactive/inflammatory cystitis conditions.
(2) There was no statistical significant correlation between MMP-9 expression and tumor stage, grade, and histological type.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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