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Research Article

Some Matrix Metalloproteases as Non-Invasive Biomarkers for Bladder Cancer in Egyptian Patients

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ABSTRACT

Bladder cancer is the most common malignancy among Egyptian males; early diagnosis and prompt treatment have been shown to improve survival at both initial diagnosis and recurrence. In the present study we aim to investigate the role of MMP-7, MMP-9 and TIMP-1 in bladder cancer progression and their relation to clinicopathological features. The study was conducted on 62 patients with bladder cancer beside 30 healthy control subjects. The levels of MMP-7, MMP-9 and TIMP-1were measured in sera of all patients before treatment and healthy controls using enzyme-linked immunosorbent assay kit (ELISA). Our results showed that serum MMP-7 and MMP-9 levels were significantly higher in cancer patients than control group ($p<0.001$) and increased according to stage, size of tumor, more lymph node involvement and metastatic disease. In contrast, serum TIMP-1 levels were lower ($p<0.001$).

In conclusion; these markers might play role in the process of cancer invasion and metastasis, but further studies are needed to determine how these markers can adequately assign patients to prognostic subgroups for different treatments and how it can be used in the design of clinical trials.

Keywords: Bladder cancer, MMP-9, MMP-7, TIMP-1

INTRODUCTION

Bladder cancer is the ninth most common cancer throughout the world and is considerably more common in developed than developing countries[1]. Bladder cancer is the most common malignancy among Egyptian males and previously has been attributed to Schistosoma infection, a major risk factor for squamous cell carcinoma (SCC), although, transitional cell carcinoma (TCC) incidence has been increasing while SCC has declined[2]. Bladder cancer is a highly prevalent and lethal malignancy, early diagnosis and prompt treatment have been shown to improve survival at both initial diagnosis and recurrence. Widespread screening has been shown to decrease mortality from bladder cancer[3]

Bladder cancer tumor markers remains a rapidly evolving field, a vast number of tumor markers have been identified and rigorously evaluated in attempts to improve non-invasive diagnostic accuracy of bladder cancer. A newer set of proteomic markers are matrix metalloproteases (MMPs) which have been studied for many years[4,5], recent advances in proteomics have allowed for more specific evaluation and identification of MMP related complexes and their utility in bladder cancer. Roy et al., reported on their findings of tumor specific urine MMP complexes including the MMP-2, MMP-9/TIMP-1 complex, MMP-9 dimer, and ADAMTS-7. They report that using a combination of MMP-2 and MMP-9 dimer in multivariate regression and binary analyses can statistically significantly differentiate bladder cancer from controls, also MMP-7 has been found to be over expressed in several tumors including bladder cancer, it is produced by stromal cells (macrophages, fibroblasts and endothelial cells) and also by tumor cells[6].

The matrix metalloproteinase's (MMPs) are a large family of zinc –dependent endopeptidases with proteolytic activity. Their activity can be regulated by various factors such as NF-KB and oxidative stress. The strongest evidence of such activity has been in vitro experiments concerning MMP-7, which has been found to be over expressed in several tumors, and it is included in the evaluation of cancer invasion and metastases[7]. Tumor cells have the capacity to produce and release matrix metalloproteinase -9 (MMP-9) a proteolytic enzyme capable of degrading basement membrane and type IV collagen in cells that is needed for tumor invasion and metastases. It may regulate angiogensis in cancer, both positively through its ability to mobilize or activate pro-angiogenic factors and negatively through generation of angiogenesis inhibitors[8].

MMPs activities are regulated by two major types of endogenous inhibitors; alpha 2 macroglobulin and tissue inhibitors of MMPs (TIMPs). TIMPs are a family comprising four members, their transcription is regulated by cytokines and growth factors. TIMP-1 is reported to mediate many complicated effects in the growth and regulation of angiogenesis in progress of tumors[9,10].

In the present study we aimed to investigate the role of MMP-7, MMP-9 and TIMP-1 in bladder cancer

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Table 1: Patients characteristics

Clinicopathological variables	Patients	controls
Number	62	30
Age (years)	61± 12	58± 14
Sex		
M/F	50/12	18/12
Smoking		
+ve /-ve	45/17	20/10
TNM		
T1/T2	7/15	---
T3/T4	15 /25	---
Node involvement	7/23/32	
N0/N1/N2		---
Distant metastases		
M0/M1	37 /25	---
Pathology		
TCC/SCC	32/30	---
Tumor size		
<3cms	20	---
>3cms	42	---

TCC = transitional cell carcinoma
SCC = squamous cell carcinoma

progression and their correlation with clinico-pathological features of disease to determine whether they could be used as a prognostic markers.

PATIENTS AND METHODS

Serum samples from 62 patients with bladder cancer were collected before treatment from Department of Surgical Oncology at National Cancer Institute. Thirty-two patients were diagnosed histopathological with transitional cell carcinoma (TCC) and 30 with squamous cell carcinoma (SCC). Tumors were staged according to TNM classification of the International Union Against Cancer Criteria (IUCC)[11] and graded according to criteria recommended by the World Health Organization[12]. For further staging, patients were diagnosed by abdominal ultrasound, intravenous pyelography, computed tomography, magnetic resonance imaging, and/or bone scans in addition to the histopathological obtained diagnosis. Thirty healthy control subjects who underwent cystoscopy for a variety of noncancerous reasons were included in the study. The local ethics committee approved the study and informed consents were taken from all patients.

The levels of MMP-7, MMP-9 and TIMP-1were measured in sera using enzyme-linked immunosorbent assay kit (ELISA) R&D systems, MN, USA.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows version 20 (IBM Corp, Armonk, NY). Data were analyzed using Student's *t*-test and analysis of variance (ANOVA) were used to address differences between patients and controls. P<.05 was considered significant.

RESULTS

Table 2: Serum levels of MMP-7, MMP-9 and TIMP-1 in cancer bladder patients and healthy controls (mean± SD (ng/ml))

Variables	Patients	Control	p-value
No.	62	30	
MMP-7	3205 ± 560	650 ± 120	<0.0001
MMP-9	5650 ± 2100	1004 ± 112	<0.0001
TIMP-1	45 ± 13	98 ± 22	<0.0001

P value <0.05 considered significant

Table (1) shows the clinicopathological characteristics of the studied groups (the sixty two patients and the healthy control group). Table (2) shows the serum levels of MMP-7, MMP-9 and TIMP-1 in cancer bladder patients and control group. Serum MMP-7 and serum MMP-9 were significantly increased (P<0.0001) in patient group comparison to control group, while serum TIMP-1 was significantly decreased (P<0.0001) in patient group in comparison to control group. Table (3) shows the seum levels of MMP-7, MMP-9 and TIMP-1 in cancer bladder patients according to clinicopathological features. MMP-7 and MMP-9 were significantly higher in patients with advanced tumor stage and size. They were higher in patients with more lymph node involvement and metastatic disease (P<0.001). Serum TIMP-1 was significantly decreased in patients with advanced tumor stage, size, more lymph node involvement and metastatic disease (P<0.001).

DISCUSSION

There are many markers associating with the progression of bladder carcinoma, such as depth of invasion, stage and multiplicity. Unfortunately they are inaccurate, which is why more clinical prognostic markers are needed. These markers could help in selection of patient management. Many markers are being studied to find more predictive markers, but it is not easy to translate these laboratory findings into a clinical instrument[13].

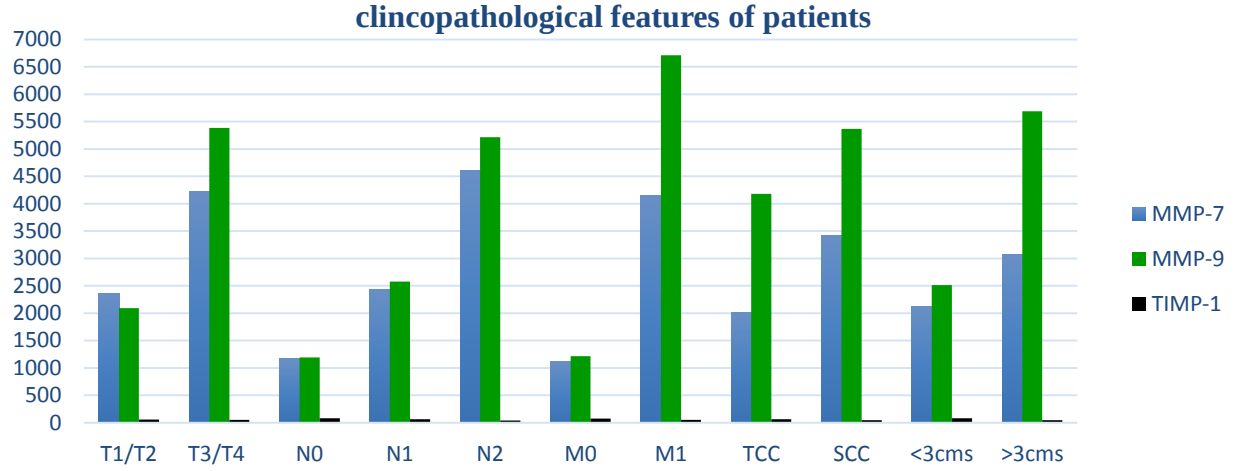
In our study we assessed the serum levels of MMP-7, MMP-9 and TIMP-1 in cancer bladder patients in relation to their clinicopathological features; MMP-7 and MMP-9 were increased according to stage while TIMP-1 was decreased according to stage. These results were in accordance with Szarvas et al.[14,15,16], they reported elevated MMP-7 concentration in urine samples and also in plasma samples from patients with bladder cancer. In urine samples no significant difference was detected in MMP-7 levels between bladder cancer patients and controls, suggesting that only MMP- 7 plasma levels could be a putative biomarker for the diagnosis of bladder cancer. Moreover, MMP-7 plasma levels may be used to identify patients at high risk of diseases progression. It is a small protein in the MMP family that lacks a C-terminal hemopexin domain common to other MMP members. MMP-7 concentration may play a role in tumor progression, as tumor invasion and progression are a multifactorial process promoted by microenvironmental changes that include overexpression of matrix

Table 3: Serum levels of MMP-7, MMP-9 and TIMP-1 in cancer patients according to clinicopathological features

Variables	MMP-7 (ng/ml)	MMP-9 (ng/ml)	TIMP-1 (ng/ml)
TNM			
T1/T2	2367± 560	2090 ± 457	56± 11
T3/T4	4212± 687	5386 ± 751	49± 15
Node involvement			
N0	1167± 122	1189±154	78± 23
N1	2435± 375	2577± 213	63± 9
N2	4602±566	5213 ± 345	41± 12
Distant metastases			
M0	1123± 66	1212± 126	71± 11
M1	4156 ± 675	6712 ± 555	52± 5
Pathology			
TCC	2014± 478	4176± 232	62± 10
SCC	3416 ± 451	5366± 672	43± 12
Tumor size			
<3cms	2119± 321	2510± 321	77± 14
>3cms	3070± 246	5689± 481	45± 12

TCC = transitional cell carcinoma; SCC = squamous cell carcinoma

Fig 1. : Serum levels of the studied parameters according to the clincopathological features of patients



metalloproteinase. Data clearly challenge the classic dogma that MMPs promote metastasis only by modulating the remodeling of extracellular matrix. Indeed, MMPs have also been attributed as an impact on tumor cell behavior in vivo as a consequence of their ability to cleave growth factors, cell surface receptors, cell adhesion molecules, and chemokines/cytokines[17].

Stetler-Stevenson[18] indicated that MMP-9 was strongly expressed in tumors that displayed recurrence compared with those that did not. It is needed in degradation of extracellular matrix and basement membranes which is essential for tumor progression in contrast to Sternlicht et al.,[19] who stated that MMP-9 positivity did not correlate to the grade or stage of the tumor, or the sex or age of the patients with bladder cancer. MMP-9 (Gelatinase B, 92kDa type IV collagenase) was first purified from human macrophages. 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[20]. 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[21].

In our work TIMP-1 was decreased in patient group in contrast to controls, this was in agreement with Gunes[22] who stated that TIMP-1 serum level was lower in bladder cancer patients according to stage and found that reduction in TIMP-1 levels may designate an increase in proteolytic activity. TIMPs, the endogenous tissue inhibitors of matrix metalloproteinase, regulate the MMP activity, but they have also been found to modulate tumor angiogenesis. It seems that MMPs have a complex role in this process, and that certain MMPs also take part in the inhibition of revascularization[17]. It has been shown that TIMPs regulate MMP activity. Findings have demonstrated that TIMPs also inhibit the mitogenic activity of human microvascular endothelial growth factors, such as VEGF-A and FGF-2. Stetler-Stevenson[23] have demonstrated a cell surface signaling receptor for a member of the TIMP family. They suggest that TIMP-2 regulates cellular responses to growth factors. This observations show that TIMP-s has other functions besides inhibition of MMPs and that it is independent of MMPs.

CONCLUSION

The prognosis of bladder carcinoma is still mostly being determined by stage, grade and multiplicity of the tumor in the bladder. In this study we investigated potential prognostic value of MMP-7, MMP-9 and TIMP-1 in bladder cancer progression. Our findings suggest that these markers might play role in the process of cancer invasion and metastasis, but further studies are needed to determine how these markers can adequately assign patients to prognostic subgroups for different treatments and how it can be used in the design of clinical trials.

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