

Prognostic Value of Circulating Matrix Metalloproteinase-2 (MMP-2) and Tissue Inhibitor of Metalloproteinase-2 (TIMP-2) in Human Bladder Cancer

¹El Baz, H., ¹Kamel, M., ²Ganzouri, H., ¹Salah, F. and ¹Zoheiry, M.

¹Immunology and ²Urology Departments, Theodor Bilharz Research Institute, Giza, Egypt

Abstract: Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play an important role in the metastasis and invasiveness of bladder cancer. The balance of secreted MMPs and their specific inhibitors is crucial in maintaining connective tissue homeostasis in physiological conditions. In neoplastic diseases, an unbalance of MMPs and TIMPs is supposed to be linked to the invasive character of tumor cells. This study was designed to evaluate the role of serum MMP-2 and TIMP-2 as non-invasive prognostic parameters in bladder carcinoma. Sixty seven patients were included in the study; 28 with chronic cystitis (11 schistosomal and 17 non-schistosomal) and 39 with malignant bladder lesions [12 schistosomal squamous cell carcinoma (Sqcc) and 27 transitional cell carcinoma (TCC) of whom 15 were schistosomal and 12 were non-schistosomal]. TCC cases were stratified according to their histopathological stage into: superficial TCC (13 cases) and invasive TCC (14 cases). Thirteen healthy individuals served as controls. Serum levels of both MMP-2 and TIMP-2 were measured by enzyme immunoassay (sandwich ELISA). All the malignant cases of the study showed a highly significant increase in MMP-2 serum levels with a corresponding highly significant decrease in their TIMP-2 serum levels when compared to both control and chronic cystitis cases ($p < 0.001$). On stratifying TCC cases according to their histopathological grade and stage; serum levels of MMP-2 were significantly increased while TIMP-2 levels were significantly decreased in association with the increase in grade ($p < 0.01$ & $p < 0.001$ respectively) and stage ($p < 0.001$, $p < 0.05$ respectively). Meanwhile, the ratio of MMP-2/TIMPs was progressively increasing with a high significance with the progress in grade and stage in TCC cases. In conclusion, High serum levels of MMP-2 and low levels of TIMP-2 exist in malignant bladder carcinoma and these levels are proportional to TCC grading and staging which may have a prognostic value. Moreover, the MMP-2/TIMP-2 ratio may play a more significant role in the determination of aggressiveness and clinical out come of bladder cancer.

Key words: Human bladder cancer, circulating matrix metalloproteinase-2 (MMP-2), tissue inhibitor.

INTRODUCTION

In Egypt, Carcinoma of the urinary bladder is the most common cancer in men constituting about 30% of all cancers (Helal *et al.*, 2006). It is characterized by high frequency of squamous cell carcinoma due to schistosomiasis which induces squamous metaplasia of the urothelium (Mostafa *et al.*, 1999). However, the frequency of transitional cell type in schistosomiasis-associated bladder cancer has been increased in the last decade (Gad EL – Mawala *et al.*, 2000). Bladder cancer is usually divided into two categories; superficial tumor (Ta & T1), which is in 75-80% of cases confined to the mucosa, and muscle invasive tumors (T2 & T3). Seventy percent of superficial bladder carcinomas show recurrence after treatment, from which, unfortunately, 30% progress to muscle-invasive tumors (Stein *et al.*, 1998). The outcome for patients with invasive disease at presentation remains poor, with distant metastasis occurring in over 50% within 2 years and an average 5-year survival of only 50% (Busch and Algaba, 2002).

One of the essential alterations that occur in malignancy is tissue invasion and metastasis (Hanahan and Weinberg, 2000). Tumor growth involves alteration in stromal extracellular matrix, and malignant tumors often induce a fibroproliferative response in adjacent stroma characterized by increased expression of type I and type III collagens. The formation of tumor stroma is often viewed as a non specific host attempt to wall off the tumor and it is thought to have a negative effect on tumor progression (Stetter-stevenson *et al.*, 1993).

Corresponding author: Hossam El-Ganzouri, Urology Departments, Theodor Bilharz Research Institute, Giza, Egypt.
E-mail: hossam_elganzouri@yahoo.com

Degradation of the basement membrane and the extracellular matrix (ECM) is a prerequisite for tumor invasion. Matrix metalloproteinases (MMPs) belong to the group of ECM degradation enzymes. The balance of secreted MMPs and their specific inhibitors (TIMPs) plays an important role in maintaining connective tissue homeostasis in normal tissue (polette *et al.*, 2004). In neoplastic diseases an imbalance of MMPs and TIMPs, leading to an excess of degradative activity, is supposed to be linked to the invasive character of tumor cells (Liotta *et al.*, 1991 and polette *et al.*, 2004). In urothelial carcinoma, several of the well characterized MMPs including MMP-2, MMP-9 and MMP-14, demonstrate increased expression and activity (Kanda *et al.*, 2000; Hara *et al.*, 2001).

It has been reported that TIMP-2 mainly forms a complex with Pro MMP-2 and could inhibit the enzymatic activity of this enzyme (lokeswar *et al.*, 1999). Therefore, the secretion ratio of MMP to TIMP play an essential role in the determination of the aggressiveness and prognosis of bladder cancer (Kexin *et al.*, 2002).

On the basis of the concept that MMPs are synthesized in tissue and released into the blood stream, this study was designed to evaluate the possibility of using MMP-2 and TIMP-2 serum levels and ratio as non-invasive prognostic markers for the clinical behavior and aggressiveness of human bladder carcinoma.

MATERIALS AND METHODS

This study included 67 urological patients (53 males and 14 females) with age range (17-45 years). All patients were referred from the urology department in TBRI. Thirteen healthy individuals (8 males and 5 females) with age range 20-48 years served as controls. Patients were subjected to detailed history taking, complete clinical examination, full routine laboratory investigations; abdomino-pelvic ultrasonography, intravenous urography, (computed tomography in selected patients), cystoscopy, transurethral resection of any bladder lesion and routine histopathological examination of the bladder biopsy specimens. Diagnosis of schistosomal infestation was based on detection of schistosomal eggs in urine or tissue with detection of circulating anti-schistosomal antibodies in sera of patients by enzyme immunoassay.

Accordingly Patients Were Classified Into:

Chronic Cystitis (Ch Cyst) Group: (28 cases)

According to Schistosomal Infestation this Group Was Subdivided Into:

- Chronic schistosomal cystitis (Ch sch cyst) (11 cases)
- Chronic non-schistosomal cystitis (Ch nonsch cyst) (17 cases)

Malignant Group: (39 cases)

According to Histopathological Diagnosis They Were Divided Into:

- Squamous cell carcinoma (SqCC). (12 cases)
- All cases were infested with schistosomiasis
- Transitional cell carcinoma (TCC) (27 cases)

TCC Group Was Subdivided According to Schistosomal Infestation Into:

- Schistosomal TCC (sch TCC) (12 cases)
- Non schistosomal TCC (nonsch TCC) (15 cases)

Histopathological Examination:

Tissue sections were fixed in 10% buffered formalin, paraffin embedded and processed routinely. Serial sections 5 µm thick were taken on poly L- lysine coated slides. Hematoxylin and Eosin stained slides were used to evaluate the pathological diagnosis of all bladder lesions, and to assess TCC cases for pathological grades as outlined by Hanham (1991) and Rosai and Ordenez (1996).

Enzyme Immunoassay for Serum MMP-2 and TIMP-2:

MMP-2 and TIMP-2 serum levels were measured by sandwich ELISA technique using commercially available kits (Quantiken MMP-2 and TIMP-2 immunoassay, R&D system, Inc. USA). Polyclonal antibodies specific for MMP-2 and TIMP-2 had been pre-coated onto a microplate. Standards and samples were pipetted into the wells, and MMP-2 or TIMP-2 was bound by the immobilized antibody. After washing away unbound substances, an enzyme linked polyclonal antibody specific for MMP-2 or TIMP-2 was added to the wells.

The ratio of MMP-2 to TIMP-2 in invasive TCC was found to be significantly higher than that in superficial TCC ($p < 0.001$). Also, this ratio was observed to be significantly increased with the progress in tumor grade ($p < 0.01$) (Table 2).

Discussion:

Proteolytic degradation of ECM is a fundamental aspect of cancer development and a key event in the regulation of tumor proliferation and metastasis. Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases that are collectively capable of degrading most components of the basement membrane and ECM and disrupting local tissue architecture to facilitate cell migration, allow tumor growth and break down basement membrane barriers for cancer invasion and metastasis (Gontero *et al.*, 2004). MMPs are secreted as inactive proenzymes and are transformed into active forms after cleavage of a propeptide domain of the molecule. They are tightly regulated at various levels, including expression level, latent form activation, and the balance between enzyme levels and their inhibitors (TIMPs). The balance between MMPs and TIMPs is critical in maintaining the integrity of ECM and its regulatory role in organ development, cell growth and differentiation (El-Badry *et al.*, 2007).

In the present study, serum levels of MMP-2 were significantly increased while TIMP-2 levels were significantly decreased in the ch cyst gp compared to control gp. Many authors had mentioned the upregulation of MMPs expression during chronic inflammation (Gomez *et al.*, 1999 and Shen *et al.*, 2001). Blood levels of MMPs and TIMPs are considered as a reflection to the presence of these enzymes in tissues as found by Sier *et al.*, 2000 and Staak *et al.*, 2006.

Sanders *et al.*, 2004 reported that MMPs have been firmly linked to inflammation and the essential step in their activation is the cleavage by proteases derived from activated inflammatory cells, especially neutrophils and monocytes. Gene expression of MMP-2 was shown to correlate in different stages with inflammation (Gomez *et al.*, 1999), also pro-inflammatory cytokines have been shown to induce transcription and expression of several MMPs (Horstrup *et al.*, 2002).

Interaction between tumor cells and matrix components are important for the growth and invasion of malignant tumors (Sier *et al.*, 2000).

The data of this study have revealed a significant increase in MMP-2 serum levels together with significant reduction in TIMP-2 levels in the malignant gp compared to both ch cyst and control gps. Comparable results were obtained by Wallard *et al.* (2006) they demonstrated increased levels of MMP-2 and MMP-9 in bladder cancer tissue as they regulate tumor development, metastasis and promote the invasiveness of malignant cells. Expression of MMPs was found by Daveis *et al.* (1993), Okada *et al.* (1994) and Kexin *et al.* (2002) in both normal bladder and Bladder cancer tissues with significant difference in the quantity of MMPs expression between the two kinds of tissues.

The balance between production and activation of MMPs and their inhibitors TIMPs is a critical aspect of cancer invasion and metastasis (Zucker *et al.*, 1999). In this work, in TCC cases, mean serum level of MMP-2 were significantly increased while, mean serum levels of TIMP-2 were significantly reduced with progress in both grades & stages of malignancy. Similar results were obtained by Vasala *et al.* (2003) who concluded that MMP-2 was revealed to serve as tissue indicator of aggressiveness and poor clinical outcome. Significant high expression of MMP-2 in bladder tumor line was found by Kallakury *et al.*, 2001 and an elevated trend of expression was observed with increased staging and grading of bladder cancer. Kexin *et al.* (2002) reported that increased level of MMP-2 was correlated with the prognosis of bladder cancer. TIMP-2 is involved in regulation of apoptosis and is associated with an adverse prognosis in patients with TCC of the bladder as reported by Gukiopoulou *et al.* (2003). Gohji *et al.* (1998) found elevated serum concentrations of MMP-2 and MMP-3 in patients with advanced TCC of the bladder in comparison to their levels in patients with superficial tumors. Over expression of MMP-2,3,13,14 promotes, while over expression of TIMPs inhibits the invasion of cancer cell lines (Kanayama, 2001).

In the present study, the ratio of MMP-2/TIMP-2 was found to be significantly increasing with the progress in tumor grade and stage. Xu *et al.* (2002) reported that when MMP-2 / TIMP-2 levels are unequal, either due to increased MMP-2 with less TIMP-2 or without changes of TIMP-2, they reliably predict aggressiveness and this ratio is highly correlated with the aggressiveness of bladder cancer .

In summary, the results of our study suggest that, serum levels of both MMP-2 and TIMP-2 may be used as non-invasive markers for the prediction of tumor behavior and its clinical outcome in patients with bladder cancer. The MMP-2 to TIMP-2 ratio may play a more significant role in the determination of the aggressiveness and metastatic potentials of the tumor.

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REFERENCES

- Busch, C. and F. Algaba, 2002. The WHO/ISUP 1998 and WHO 1999, systems for malignancy grading of bladder cancer. Scientific foundation and translation to one another and previous systems. *Virchows Arch.*, 441: 105-108. *Cell*, 100: 57-70.
- Davies, B., J. Waxman, H. Wasan, P. Abel, G. Williams, T. Krausz, D. Neal, D. Thomas, A. Hanby, F. Balkwill, 1993. Levels of matrix metalloproteinases in bladder cancer correlate with tumor grade and invasion. *Cancer Res.*, 53: 5365-5369.
- El Badry, A.A., A. Abou El-Fadle, A.L. El-Balshy, 2007. Tissue Inhibitor of Matrix Metalloproteinase-2 in Nasopharyngeal Carcinoma. *Medscape General Medicine. Eur. Urol.*, 46: 296-311.
- Gad El-Mawla, N., M.N. El-Bolkainy, H.M. Khaled, 2000. Bladder cancer in Africa: update, *Semin. Oncol.*, 28: 178-188.
- Gakiopoulou, H., L. Nakopoulou, A. Siatelis, I. Mavrommatis, E.G. Panayotopoulou, I. Tsirmpa, C. Stravodimos, A. Giannopoulos, 2003. Tissue inhibitor of metalloproteinase-2 as a multifunctional molecule of which the expression is associated with adverse prognosis of patients with urothelial bladder carcinomas. *Clin. Cancer Res.*, 9: 5573-5581.
- Gohji, K., N. Fujimoto, J. Ohkawa, A. Fujii, M. Nakajima, 1998. Imbalance between serum matrix metalloproteinase-2 and its inhibitor as a predictor of recurrence of urothelial cancer. *Br J Cancer*, 77: 650-655.
- Gomez, D.E., M.S. Lorenzo, D.F. Alonso, Z.A. Andrade, 1999. Expression of metalloproteinases (MMP-1, MMP-2 and MMP-9) and their inhibitors (TIMP-1 and TIMP-2) in schistosomal portal fibrosis. *Am. J. Trop. Med. Hyg.*, 6: 9-13.
- Gontero, P., S. Banisadr, B. Frea, M. Brausi, 2004. Metastasis markers in bladder cancer: a review of the literature and clinical considerations. *Eur. Urol.*, 46: 296-311.
- Hanahan, D., R.A. Weinberg, 2000. The hallmarks of cancer. *Cell*, 100: 57-70.
- Hanham, I.W.F., 1991. Cancer of the kidney and urinary tract In: International Union Against cancer (UICC). *Manual of Clinical Oncology*. Hossfeild, D.K., Shermann C.D., Lone R.R. (eds). 5th edition, Berlin, Springer Verlag., 311-318.
- Hara, I., H. Miyake, S. Hara, S. Arakawa, S. Kamidono, 2001. Significance of matrix metalloproteinases and tissue inhibitors of metalloproteinase expression in the recurrence of superficial transitional cell carcinoma of the bladder. *J. Urol.*, 165: 1769-1772.
- Helal, Tel A., M.T. Fadel, N.K. El-Sayed, 2006. Human papilloma virus and p53 expression in bladder cancer in Egypt: relationship to schistosomiasis and clinicopathologic factors. *Pathol. Oncol. Res.*, 12: 173-178.
- Horstrup, J.H., M. Gehrman, B. Schneider, 2002. Elevation of serum and urine levels of TIMP-1 and tenascin in patients with renal disease. *Nephrol. Dial. Transplant*, 17: 1005-1013.
- Kallakury, B.V., S. Karikhalli, A. Haholu, C.E. Sheehan, N. Azumi, J.S. Ross, 2001. Increased expression of matrix metalloproteinases 2 and 9 and tissue inhibitors of metalloproteinases 1 and 2 correlate with poor prognostic variables in renal cell carcinoma. *Clin. Cancer Res.*, 7: 3113-3119.
- Kanayama, H., 2001. Matrix metalloproteinases and bladder cancer *J. Med. Invest.*, 48: 31-43. .
- Kanda, K., M. Takahashi, Y. Murakami, H. Kanayama, S. Kagawa, 2000. The role of the activated form of matrix metalloproteinase-2 in urothelial cancer. *B.J.U. Int.*, 86: 553-557.
- Kexin, X.U., H.O. Shukun, D.U. Zhijun, 2002. Prognostic value of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 in bladder carcinoma. *Chin. Med. J.*, 115: 743-745.
- Liotta, L.A., P.S. Steeg, W.G. Stetler-Stevenson, 1991. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell*, 64: 327-336.
- Lokeshwar, B.L., 1999. MMP inhibitions in prostate cancer. *Ann. N.Y. Acad. Sci.*, 30: 271-289.
- Mostafa, M.H., S.A. Sheweita, P.J. O'Connor, 1999. Relationship between schistosomiasis and bladder cancer. *Clin. Microbiol. Rev.*, 12: 97-111.
- Okada, Y., 1994. A matrix metalloproteinase expressed on the surface of invasive tumor cells. *Nature*, 370: 651-652.
- Polette, M., B. Nawrocki-Raby, C. Gilles, C. Clavel, P. Birembaut, 2004. Tumor invasion and matrix metalloproteinases. *Crit. Rev. Oncol. Hematol.*, 49: 179-186.

Rosai, J. and N.G. Ordonez, 1996. Urinary tract In: Acherman: Surgical Pathology. Rosa J., Gery L. and Joiner P (eds), Patterson AS and Van Hoffmann Press, Beaumont Book, division in USA, 1190-1210.

Sanders, J.F., H.V. Goor, R. Hanemaaijer, C.G. Kallenberg, C.A. Stegeman, 2004. Renal expression of matrix metalloproteinases in human ANCA- associated glomerulonephritis. *Nephrol. Dial. Transplant.*, 19: 1412-1419.

Shenx, Y., Y. Kobayashi, F. Zhao, H. Okunishi, 2001. Up-regulated expression of matrix metalloproteinases in the chronic inflammation. *Zool. Sci.*, 18: 114-119.

Sier, C.F., C. Giovanni, J.H. Verheijen, A. Tizzani, V. Agape, J. Kos., F. Blasi, R. Hanemaaijer, 2000. Enhanced urinary gelatinase activities (matrix metalloproteinases 2 and 9) are associated with early stage bladder carcinoma: A comparison with clinically used tumor markers. *Clin. Canc. Res.*, 6: 233-234.

Staak, A., S. Badendiek, D. Schnorr, S.J. Loening, K. Ung, 2006. Combined determination of plasma MMP2, MMP9, and TIMP1 improves the non-invasive detection of transitional cell carcinoma of the bladder. *B.M.C. Urol.*, 10: 6-19.

Stetter-stevenso, W.G., S. Aznavooring, L.A. Liotta, 1993. Tumor cell interaction with the extracellular matrix during invasion and metastasis. *Annu Rev. Cell Biol.*, 9: 541-573.

Stein, J.P., G.D. Grossfeld, D.A. Ginsberg, D. Esrig, J.A. Freeman, D.G. Skinner, R.J. Cote, 1998. Prognostic markers in bladder cancer: A contemporary review of the literature. *J. Urol.*, 160: 645-659.

Vasala, K., P. Paakko, T. Turpeenniemi-Hujanen, 2003. Matrix metalloproteinase-2 immunoreactive protein as a prognostic marker in bladder cancer. *Urology*, 62: 952-957.

Wallard, M.J., C.J. Pennington, A. Veerakumarasivam, G. Burt, I.G. Mills, A. Warren, H.Y. Leung, G. Murphy, D.R. Edwards, D.E. Neal, J.D. Kelly, 2006. Comprehensive profiling and localisation of the matrix metalloproteinases in urothelial carcinoma. *Br. J. Cancer*, 94: 569-577.

Xu, K., S. Hou, Z. Du, 2002. Prognostic value of matrix metalloproteinase-2 and tissue inhibitor of metalloproteinase-2 in bladder carcinoma. *Chin. Med. J. (Engl)*, 115: 743-751.

Zucker, S., M. Hymowitz, C. Conner, H. Zarrabi, N. Hurewitz, L. Matrisian, D. Boyd, 1999. Measurement of Matrix Metalloproteinases and Tissue Inhibitors of Metalloproteinases in Blood and Tissues: Clinical and Experimental Applications. *Ann New York Acad. Sci.*, 878: 212-227.