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Study of Correlation between Prostate Specific Antigen and Matrix Metalloproteinase-2 Activity in Benign and Malignant Prostate Hyperplasia

Nastaran Alizadeh[1], Omid Safa[1], Mohammad Reza Khorramizadeh[*2] and Mohammad Pezeshki[2]

[1]*Div. of Immunology, Dept. of Pathobiology, School of Medicine, Hormozgan University of Medical Sciences, Hormozgan;* [2]*Dept. of Pathobiology, School of Public Health and Institute of Public Health Research, Tehran University of Medical Sciences, Tehran, Iran.*

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ABSTRACT

There are emerging data on novel tumor markers such as matrix metalloproteinase-2 (MMP-2 or gelatinase-A), which play a key role in tissue invasion and metastasis. We designed an investigation to assess the usefulness of MMP-2 activity as compared to prostate specific antigen (PSA) in cancer staging process. We have analyzed the circulating form of MMP-2 in serum samples of patients suffering from either benign prostate hyperplasia (BPH, n = 54) or prostate cancer (PC, n = 26), and prostatitis (n = 4) as compared to control normal individual (n = 26), respectively. Protein-content adjusted samples were separated by gelatin-embedded polyacrylamide gel electrophoresis then were subjected to densitometric analysis. Total PSA (tPSA) and free PSA (fPSA) were quantified using a standard ELISA technique. Correlation coefficient (r) between tPSA and MMP-2 activity in patient group was +0.938 and in controls group was 0.799 (*P*<0.01). In addition, (r) between tPSA and MMP-2 activity in PC patients was 0.940 and in BPH patients was 0.962 (*P*<0.01). (r) between fPSA and MMP-2 activity in PC patients was 0.913, in BPH patients was 0.644, and in prostatitis patients was -0.994 (*P*<0.01). These results demonstrate that MMP-2, compared to PSA, might be considered as a better tumor marker in monitoring and screening patients with PC. *Iran. Biomed. J. 10 (1): 27-32, 2006*

Keywords: Matrix metalloproteinase-2 (MMP-2), Prostate Hyperplasia, Prostate Specific Antigen (PSA), Zymography

INTRODUCTION

Prostate cancer (PC) is the second most common cause of cancer death in men in the world. The most threatening and primary cause of death from PC is invasion and metastasis. One of the first events in progression to malignancy is the degradation of basement membrane (BM) and extracellular matrix (ECM), followed by invasion, a critical early step in the metastatic cascade. When metastasis has occurred, there is no curative treatment for the patients. A correlation between the increase of the secretion of matrix-degrading serine proteases and matrix metalloproteinases (MMP) with invasion, metastasis, and aggressiveness of cancer has been demonstrated [1]. The MMP are a family of Zn^{2+} endopeptidases that can digest various ECM macromolecules. More than 200 members have been identified and classified into five major types of structurally and functionally related metalloproteinases [2, 3]. Among members of this family of human MMP, MMP-2 and MMP-9 (gelatinase A and B) degrade BM collagens type IV, gelatin and other proteoglycan component of ECM [4]. MMP have been reported to play a significant role in either physiological (e.g. aging, wound healing, and angiogenesis) or pathological (e.g. allergic contact dermatitis, tumor invasion, and metastasis) processes [5-7].

Recent reports have indicated that MMP-2 plays a key role in the proteolytic cascade leading to ECM

*Corresponding Author; Tel. (+98-21) 6646 2268; Fax: (+98-21) 6646 2267; E-mail: khoramza@sina.tums.ac.ir

Abbreviations: BPH, benign prostate hyperplasia; **MMP**, matrix metalloproteinases; **PSA**, prostate specific antigen; **PC**, prostate cancer ; **fPSA**, free prostate specific antigen ; **tPSA**, total prostate specific antigen; **(r)**, correlation coefficient ; **BM,** basement membrane; **ECM**, extracellular matrix ; **HRP**, horse radish proxidase

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degradation during tumor invasion and metastasis, in particular, to be specifically associated with PC metastasis [8-13]. Since molecules released as the results of tissue alterations are often found in body fluids, the determination of MMP in blood or urine has been recommended as diagnostic measure to characterize the processes occurring in tissue [14]. Numerous methods have been developed to assess the levels of active and latent forms of MMP-2 in biological fluids. One of the most common methods is zymography, which is an electrophoretic technique that provides useful quantitative information on the enzyme as well as an estimation of molecular weight [15, 16]. In the present study, we used zymography technique to assess the circulating form of MMP-2 in 24 cases of PC vs. 50 serum samples of benign prostate hyperplasia (BPH) as compared to 26 serum samples of control individuals.

MATERIALS AND METHODS

Patients. Based on pathological needle biopsies confirming the involvement of either adeno-carcinoma or BPH, 26 PC (mean prostate specific antigen [PSA] = 3.0), 54 BPH (mean PSA = 7.0) and 4 prostatitis (mean PSA = 22.0) patients were included and categorized as sub-groups in this study, respectively. Normal individuals (n = 26, mean PSA = 0.8) were also included as control group. Mean age of all sub-groups and normal individuals was 65. A written consent was taken from each patient for inclusion of his record in the study. Patient sera, taken from a 5-ml clotted blood, were aliquoted and cryopreserved in -70°C for further analyses.

MMP-2 zymography. Serum samples for analyses were prepared by dilution into zymogram sample buffer consisting of 0.4 M Tris (pH 6.8), 5% SDS, 20% glycerol, and 0.03% bromophenol blue. Moreover, as control positive (C+), we used supernatant of HT 1080 cell line, as a known reference, to show the gelatin activity of MMP-2 [15]. The volume of samples to be loaded on the wells was adjusted according to the protein content (each well contained 20 µg protein) of a precast 7% polyacrylamide gel containing 0.5% gelatin A and the electrophoresis was carried out at 20 mA constant current for 2.5 to 3.0 h, at which time the front bromophenol blue dye had reached the bottom of the gel. The gel was removed and incubated for 1 h at room temperature in 100 ml of 2.5% Triton X-100 on a rotary shaker. The Triton X-100 solution was decanted and replaced with 100 ml of enzyme buffer (50 mM Tris, pH 7.5, 200 mM NaCl, 5 mM $CaCl_2$, and 0.02% NaN_3). The gel was then incubated at 37°C over night. Staining and destaining were carried out at room temperature on a rotary shaker. Each gel was stained with 100 ml of 0.5% Coomassie blue G-250 in 30% methanol, 10% acetic acid for 1 h and then destained with three changes of 30% methanol, 10% acetic acid (for 15, 30, and 60 min of distain time, respectively, for each change). After these steps, areas of digestion were appeared as non-staining regions of the gel (Fig. 1). The degree of digestion was quantified using an UVI Pro gel documentation system (GDC-8000 System) on the basis of grey levels, calculated and depicted as "mean area density" in arbitrary unit. Each serum (PC, BPH, and Normal) was examined in triplicate and the average value of the integrated density for a particular band was used for further calculation [17, 18].

Total PSA (tPSA) and free PSA (fPSA) determination. The serum level of tPSA and fPSA was determined by solid phase, non-competitive assay based upon the direct sandwich ELISA. Fifty microliters of standards, control, and patient serum were incubated for 1 h while shaking at room temperature together with the anti PSA and anti fPSA antibodies in Streptavidin coated microtiter strips. The strips were then washed with washer solution followed by incubation with horse radish peroxidase (HRP) labelled anti PSA and anti fPSA antibodies. After washing, buffered substrate/ chromogen reagent was added to each well and the enzyme reaction was allowed to proceed and it was then incubated for 30 min at room temperature with constant shaking. Finally, 100 µl of HRP stop solution was added to each well and then absorbance was read at 405 nm in a microtiter plate reader [19, 20].

Statistics. Statistical significance of MMP-2 mean area density, fPSA and tPSA concentrations was determined by the Pearson (r), Mannwhitney U and $P<0.01$ was considered significant.

RESULTS

Gelatinolytic activity of MMP-2 on the basis of calculated mean area density in patient and in control groups was 11601.92 and 1685.51, respectively. Mean area density demonstrated that

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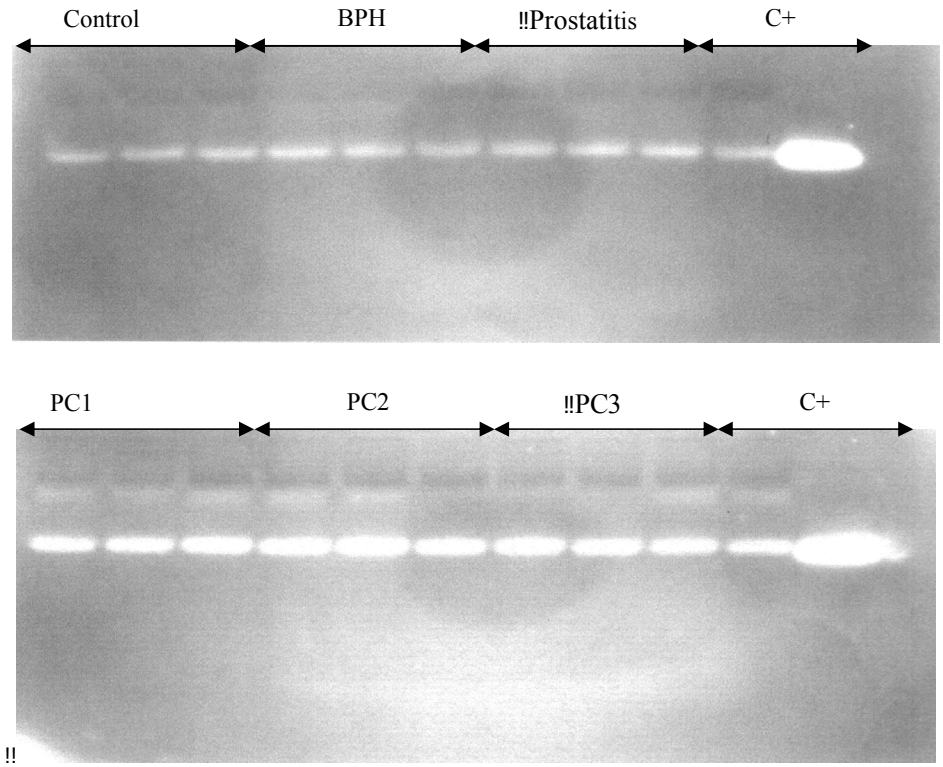

Fig. 1. Double gelatin zymogram gels of serums samples from prostate hyperplasia patients (PC, BPH, Prostatitis) and from control donors (control). We used supernatant of HT 1080 cell line as control positive (C+) to show the gelatin activity of MMP-2. PC1, PC2, and PC3 are serum samples of three patients with prostate cancer, as were shown, gelatinolytic activity of these patients was significantly higher than those have not suffering from prostate cancer or control individuals.

MMP-2 activity is significantly higher in PC vs. BPH as compared to control group (normal individuals), as depicted in Table 1 ($P<0.01$). (r) between tPSA and MMP-2 activity in all patient sub-groups (calculated altogether) as compared to control group was 0.938 and 0.799, respectively, indicating a significant association between tPSA and MMP-2 in pathologic processes rather than normal conditions, ($P<0.01$).In addition, (r) between tPSA and MMP-2 activity in PC and BPH patients was 0.940 and 0.962, respectively, demonstrating a stronger contribution of tPSA and MMP-2 in BPH patients ($P<0.01$) (Fig. 2). (r) between fPSA and MMP-2 activity in PC and BPH sub-groups was 0.913 and 0.644, respectively. This value for prostatitis sub-group was -0.994 ($P<0.01$) (Fig. 3). This recent data, altogether, advocates a sound correlation between fPSA and MMP-2 in PC vs. BPH and prostatitis sub-groups.

Table 1. Mean area density of gelatinolytic activity in patient sub-groups and control group.

	Group	Number of cases	Mean of gelatinolytic activity	Standard deviation (SD)
*Patients subgroups	PC with PSA<50 ug/l	21	16404.90	4795.24
	PC with PSA>50 ug/l	5	78831.53	17699.54
	BPH	54	3781.00	2371.74
	Prostatitis	4	7925.00	1583.86
	Total	84	11601/92	18516/97
normal control group		26	1685/5128	485/5569

Mann-Whitney U = 233, z = -6.044, $P<0.01$; *Mann-Whitney U = 44, z = -6.759, $P<0.01$.

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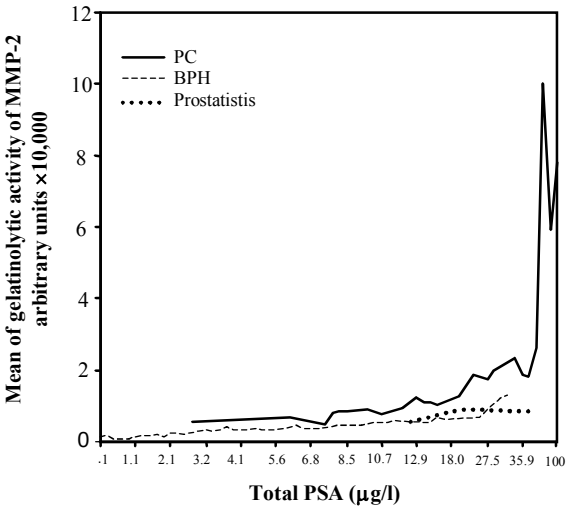

Fig. 2. Correlation between mean area density of MMP-2 activity and tPSA concentration in patient's sub-groups. TPSA concentration was plotted against mean area density of MMP-2 activity. As shown in Figure, in high level of tPSA, MMP-2 activity is higher increased specially in PC patients. PC, prostate cancer; BPH, benign prostate hyperplasia.

DISCUSSION

PC is the most common non-cutaneous malignancy in adult men in the United States. In addition, BPH is the most common non-malignant hyperplasia in men. PSA is a 33-kDa serine protease that characteristically secreted from prostatic epithelial cells and its secretion is regulated by androgens [16]. Since the discovery of PSA, detection and treatment of PC have changed dramatically. However, distinguishing between PC and non-PC (BPH and prostatitis) in subjects with PSA levels of 4.1 to 10.0 ng/ml (known as gray zone), is still controvrsial.. Some studies using PSA molecular forms at the initial biopsy, and a cut!off value of fPSA percentage between 20% and 25% in the clinical setting [21-24]. Another study demonstrated that individuals with initial biopsy (for) finding of small acinar glands suspicious to cancer and those with high-grade prostatic intraepithelial neoplasia remain at risk of PC [25]. It has been therefore proposed that more valuable information, together with other routine findings, could be employed for predicating the likelihood of PC. However, these PSA-related markers are useful to suggest prostate biopsy and avoid needless biopsy in subjects with intermediate PSA levels, but are not sufficient for staging of PC. Therefore, use of novel tumor markers seem to be necessary. (This part is not clear).

The principle objective of this study, namely, to assess the applicability of MMP-2 activity as compared to tPSA and fPSA levels in cancer screening and staging. As shown in Table 1, mean of MMP-2 activity is significantly higher in patients with PC than BPH and prostatitis patients. These data demonstrate that the probability of favorable pathologic finding is increased parallel to increasing MMP-2 activity and show a positive correlation between MMP-2 activity and adverse pathologic features, i.e. cancer aggressiveness. In addition, in gray zone (tPSA levels of 4.1 to 10.0 ng/ml), MMP-2 activity is significantly higher in PC vs. BPH and prostatitis patients (Fig. 2). In other words, mean area density of MMP-2 activity has positive correlation with fPSA level in patient sub-groups and it is significantly higher in PC patients (Fig. 3). The present study in agreement with some other reports [26-28] demonstrated that evaluation of MMP-2 is a reliable alternative biomarker for PC monitoring. Our findings indicate that determination of MMP-2 activity as compared to tPSA and fPSA could be a valuable means for screening and monitoring of the patients suffering from PC.

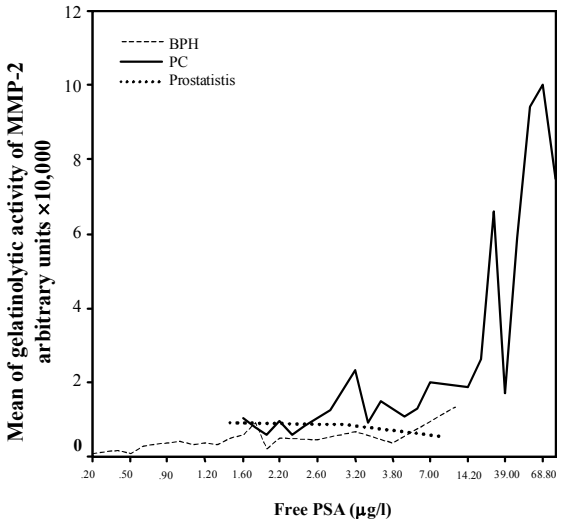

Fig. 3. Correlation between mean area density of MMP-2 activity and fPSA concentration in patients' sub-groups. The concentration of fPSA was plotted against mean area density of MMP-2 activity. As shown in the Figure, in PC patients, MMP-2 activity is highly increased. PC, prostate cancer; BPH, benign prostate hyperplasia.

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