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Original Article

Evaluation of Matrix Metalloproteinase 2 and 9 Activity in Patients with Prostate Cancer and Benign Prostate Hyperplasia Compared with Healthy Individuals

Maryam Shojaee[1], Parisa Mohammadi[2], Hamed Jafarpour[3], Somayeh Pouriamehr[4], Haleh Barmaki[5], Abbas Khonakdar-Tarsi[6*]

1. Department of Biology, Payame Noor University of Mashhad, Mashhad, Iran.
2. Department of Clinical Biochemistry, School of Medicine, Babol University of Medical Sciences, Babol, Iran.
3. Student Research Committee, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.
4. Department of Biology, Dezful Branch, Islamic Azad University, Dezful, Iran.
5. Department of Laboratory Medicine, Faculty of Para Medical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
6. Department of Biochemistry and Genetics, Immunogenetic Research Center (IRC), Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran.

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Abstract

Background and Aim: Prostate cancer (PC) is one of the most prevalent cancers with high mortality and morbidity in men, which can be treated in different ways before the progression and metastasis to distant organs. Destruction of extracellular matrix by matrix metalloproteinase (MMP), particularly by the 2 and 9 subtypes, has an important role in the metastasis of PC. We aimed to assess the activity of MMP 2 and 9 and some related metalloproteinases in PC and with benign prostate hyperplasia (BPH) patients in comparison to healthy individuals.
Methods: In this case-control study, 72 individuals referred to Imam Khomeini hospital (Tehran, Iran), have been divided into 3 groups, including PC, BPH, and healthy control. Age and body mass index (BMI) for all groups have been matched. Venous blood samples were used to assess the enzyme activity by the zymography technique.
Results: The activity of MMP-2 and 9 was significantly higher in PC than BPH and control groups. But there was no difference in the activity of enzymes in patients with PC according to the Gleason score.
Conclusion: The results suggested that MMPs activity can be considered a diagnostic marker for PC. However, further studies are required to establish this concept.

Keywords: Benign prostate hyperplasia; Metalloproteinase; Prostate Cancer.

[*]**Corresponding Author:** Abbas Khonakdar-Tarsi; **Email:** a.khonakdar@mazums.ac.ir

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Introduction

Prostate cancer (PC) is one of the most prevalent malignancies in men worldwide. In 2018, 164690 new cases of the disease have been reported in the United States. Among them, approximately 29430 patients passed away. PC contains 19.23% of all cancer morbidity and 9% of all cancer-related death in the men population worldwide (1).

This cancer can be treated by current strategies if it is limited to just prostate tissue. But, the treatment almost is not completely effective when it metastases to other tissues. The metastasis is a complex cascade process leading to tumor cell migration, attachment, and invasion. Cellular invasion contains the tumor cell translocation across the extracellular matrix barrier as a known important biological event needed for tumor metastasis (2).

The MMPs are a family of proteolytic enzymes that degrade extracellular matrix components such as collagen, fibronectin, and laminin (3). The most important component of the basal membrane is collagen IV, which is degraded by MMP-9. In this regard, the evaluation of MMP-9 proteolytic activity is essential for an understanding of basal membrane change and repair mechanisms and abnormal collagen destruction in pathologic conditions such as atherosclerosis, cancer, and rheumatoid arthritis (4-7).

Among all types of MMPs, MMP-2, 9 can degrade abnormal collagen and the types of IV, V, VII, IX. Recently their role in apoptosis, differentiation, angiogenesis, immune responses, and tumor cell growth has been revealed (8). The MMP-9 expression was associated with a higher rate of metastasis; it was confirmed that the enzyme inhibitors reduced the metastasis rate of PC (9). The MMP-9 expression was higher in the serum and tissue of PC compared to BPH (10, 11). The increasing MMP-2 expression is associated with a decreased survival rate in patients with PC (12, 13). MMP-2 also was known as an activator of MMP-9. (14). Therefore, it aimed to assess the activity of MMP-2 and 9 in PC and BPH patients in comparison to healthy men.

Methods

Among the patients who were referred to the urology center in the Imam Khomeini hospital in Tehran City during 2018-2019, Forty-eight patients were selected and after the biopsy and pathological tests were divided into two groups: PC group (n=24) and BPH (n= 24). The Third group included 24 healthy people. Exclusion criteria in the PC group include the patients who were diagnosed with more than one year of diseases and who received anti-cancer drugs, chemotherapy, hormone therapy, and radiotherapy. Inclusion criteria in the BPH group include the BPH detection and PC ruling out according to histological survey following open prostatectomy.

Exclusion criteria in the BPH group include the patients with a history of cancer, received finasteride more than one month and anti-cancer drugs, and whose prostate histological evaluation showed a section suspected to prostate intraepithelial neoplasia (PIN).

All the individuals who entered the study signed the testimonial consciously and with desire.

Blood sampling

From all individuals, about 2.5 mL of blood samples were collected into tubes without any coagulant agents from the forearm, and serum was extracted and used for zymography tests.

Zymography

Serum samples were electrophoresed on polyacrylamide gel 10% with sodium dodecyl sulfate (SDS-PAGE) and gelatin 1% (gelatinase substrate). After electrophoresis, the gel was incubated in 2% Triton X-100 solution in Phosphate-buffered saline (PBS) for one hour at room temperature and then in Tris-HCL (pH 7.4, containing 10 mmol calcium-chloride) for 16 hours at 37°C. Following washing, the gel was stained by 0.05% coomassie brilliant blue G-250. Then, the destaining was done with a solution of water: methanol: acetic acid with a ratio of 60%, 30%, and 10%, respectively. The destained bands produced by MMP-9, MMP-2, MMP-9/NGAL, and dimmer MMP-9 activity appeared in a purple background. Protein weight markers (Color Burst, Sigma Aldrich; USA) were used to confirm the identity of the gelatinase band. After the complete destaining, the gels have been filled between two transparent films and scanned by a Canon scanner (LiDE110, Japan).

Quantification of bands produced by gelatinase activation

Colorless bands of zymography gels produced by the activity of MMP-9, MMP-2, MMP-9/NGAL, and dimmer MMP-9 were quantified by measurement of the bands' area with ImageJ software.

Statistical analysis

To compare the mean activity of MMP-9, MMP-2, MMP-9/NGAL, and Dimmer MMP-9 enzymes in patients groups with the healthy group, the SPSS-20 software was used for statistical analysis and T-test, ANOVA and Tukey tests were used and the results were reported in Mean ±standard deviation (SD). Bivariate correlation test and Spearman's rho

statistical method were also used to investigate the association between marker activity and cancer stage. All results reported in 95% confidence interval (CI) and p-value <0.05 were considered significant.

Results

The demographic results were compared between three PC, BPH, and cancer (Table 1). There were no statistical differences between the three groups in age and BMI, so we considered that the groups are matched. The groups were also examined for smoking and family history. There were no statistical differences between groups according to familial history and smoking.

Table 1. Comparison of the demographic result between the three groups

Characteristics			N	Mean± SD	P-value
Age (year)	PC		24	64.00± 6.11	0.597
	BPH		24	66.63± 6.40	
	control		24	65.85±10.69	
BMI (Kg/m^2)	PC		24	23.21± 3.21	0.297
	BPH		24	22.16± 3.20	
	control		24	23.61± 3.08	
			N	**percent**	
Familial history	PC	Yes	2	8.34	0.357
		No	22	91.66	
	BPH	Yes	1	4.17	
		No	23	95.83	
	control	Yes	0	0	
		No	24	100	
Smoking	PC	Yes	7	29.17	0.163
		No	17	70.83	
	BPH	Yes	4	16.67	
		No	20	83.33	
	control	Yes	10	41.67	
		No	14	58.33	

PC: Prostate cancer; MMP: matrix metalloproteinase; BPH: benign prostate hyperplasia

It can be seen from the data in Table 2 that there is a significant difference in the quantitative activity of MMPs except for MMP-9/NGAL between the three groups. Tukey test was used to determine the difference of quantitative activity of MMPs between each group. The data shown in Table 3 demonstrated that the Dimmer MMP-9 between cancer and control groups, the MMP-9 and MMP-2 between the cancer group with the control and BPH groups differed significantly.

Table 2. Comparison of the quantified activity level of MMPs between three groups

Variables		Mean±SD	p-value
Dimmer MMP-9 (IU)	PC	163.35± 40.85	0.009
	BPH	125.86±52.77	
	control	94.31±72.98	
MMP-9/NGAL (IU)	PC	20.56±18.98	0.079
	BPH	34.42±33.59	
	control	18.13±7.70	
MMP-9 (IU)	PC	291.98±141.81	0.001
	BPH	144.90±90.77	
	control	193.96±50.25	

MMP-2 (IU)	PC	113.18±65.38	0.019
	BPH	70.59±44.23	
	control	71.24±26.33	

PC: Prostate cancer; MMP: matrix metalloproteinase; BPH: benign prostate hyperplasia

Table 3. Multiple Comparison of the quantified activity level of MMPs between each group

Enzyme	Groups		p-value
Dimmer MMP-9 (IU)	PC	control	0.006
MMP-9 (IU)	PC	BPH	0.000
		control	0.041
MMP-2 (IU)	PC	BPH	0.026
		control	0.041

PC: Prostate cancer; MMP: matrix metalloproteinase; BPH: benign prostate hyperplasia

The prognosis of men with prostate cancer evaluated by the Gleason grading system (Table 4). The prostate tissues that they have prepared by the biopsy, are evaluated by the pathologists and as cancer progresses, they give it a score of 2 to 9. The higher numbers indicate greater risks and higher mortality. The results demonstrate that when patients with PC are divided into two groups according to the Gleason score of >7 and ≤7, no differences are seen in the activity of the enzymes. The correlation analysis between different factors demonstrated that MMP-9 positively correlated with MMP-2 (R: 0.708; P < 0.001). Other factors showed no significant correlations.

Table 4. Comparison of the quantified activity level of MMPs in patients with PC based on the Gleason score

Variables	GS divided	Mean	Std. Deviation	p-value
Age (year)	>7	64.50	7.06	0.810
	≤7	63.70	5.85	
BMI (kg/m^2)	>7	22.24	1.75	0.285
	≤7	23.79	3.80	
Dimmer MMP-9 (IU)	>7	161.41	30.67	0.899
	≤7	164.57	48.16	
MMP-9/NGAL (IU)	>7	22.09	17.87	0.806
	≤7	19.64	20.50	
MMP-9 (IU)	>7	293.31	180.96	0.977
	≤7	290.99	118.13	
MMP-2 (IU)	>7	143.97	63.78	0.282

Discussion

The important processes in cancer development are angiogenesis, metastasis, and distribution of tumor cells far from the original location. The process of angiogenesis includes the growth and branching of blood vessels in tumor tissue (15, 16). Several studies illustrated that MMPs such as MMP-2 and MMP-9 have a key role in angiogenesis (17-21).

Trudel D et al. demonstrated MMP-9 is over-expressed in high-grade prostate tumor cells and also in benign stromal and epithelial cells in the early stages (22). These findings were in line with our results. In the present study, MMP-9 activity was 291.98 in patients with PC, while in the BPH and control groups, it was 144.9 and 193.96, respectively. Moreover, these results showed that MMP-9 activity significantly increased in prostate cancer. These results were not consistent with Rodrígue G *et al*. results that they didn't observe any association between the plasma expression of MMP-9 and prostate syndromes, so it can't be considered as a marker for PC diagnosis (23). According to previous studies, it can be concluded that MMP-9 isn't a proper diagnostic marker but can be considered as a target molecule for inhibiting

tumor progression. Based on current results, both MMP-2 and MMP-9 enzymes can be used as diagnostic markers because their expression is not significantly different in healthy men and benign prostatic hyperplasia, but in the group of prostate cancer with both healthy and BPH is significantly different.

Morgia G et al. investigated the MMP-13 as a diagnostic marker and MMP-2, 9 as prognostic markers in PC. They concluded that the plasma level and activity of MMPs concomitant with PSA determination can play a key role in the diagnosis, treatment, and screening of prostate cancer (11).

Wilson et al. reported that produced protease-activated receptors 1 and 2 (PAR-1 and PAR-2) can increase the activity of MMP-2, 9 in PC cells that confirm their role in PC metastasis (24). Our result consistent with Wilson's research demonstrated MMP-2 activity in patients with PC is remarkably higher than the BPH and the control group. Hamdy F.C et al. and Festuccia C et al. showed the elevation of MMP-9 levels in patients with PC compared to BPH (25, 26). But several studies reported opposite results. Lokeshwar et al. (27) revealed that BPH caused a higher level of MMP-9 in comparison to patients with PC, while the level of MMP-2 in PC samples was more than BPH (28-31). Similar studies proved an increased level of MMP-2 in PC compare to BPH, however, Upadhyay et al. (32) didn't observe any significant difference in MMP-2 expression among patients with PC and healthy control. No definitive specificity of these enzymes for cancer was found in this study, but it is confirmed that they are significantly higher in cancer patients. On the other hand, in people who have recently been diagnosed with prostate cancer, they can be used as specific therapeutic targets in the cancer site.

Conclusion

The results suggested that MMPs activity can be considered a diagnostic marker for PC. However, further studies are required to establish this concept.

Conflict of Interest

The authors declared that they have no conflict of interest.

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Ethics

The protocol of experiments was permitted by the ethical committee of MAZUMS (code: IR.MAZUMS.REC.1398.1065).

Author contributions

MM, HJ, HB, and SP performed sampling and experiments and assembled input data. AK-T and HM-S designed the study, analyzed and interpreted the data, and wrote the paper.

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