Investigating MMP-9 Gene Tissue Expression in the Diagnosis of Prostate Cancer

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Abstract: *Background and Objective by releasing growth factors, MMP-9, or gelatinase B, plays a major role in the formation and progression of tumors. Changes in MMP-9 expression on a cellular level and forms of its secretion promote prostate cancer growth and increase the rates of metastasis and angiogenesis. The purpose of this study was to examine tissue expression of MMP-9 gene in the diagnosis of prostate cancer. Methodology in this study, 63 patients were selected: 33 patients with Benign Prostate Hypertrophy (BPH) and 30 with Prostate Cancer (PCa). Fore gene expression, the RNA was extracted using TRIZOL and converted to cDNA via a reverse transcription enzyme. Finally, MMP-9 genes along with cDNA were injected into the Real-Time PCR device using a set of specifically designed primers. Results the results indicated that MMP-9 gene expression was significantly higher in patients with PCa than those with BPH (p ≤0.001). Moreover, MMP-9 gene expression was significantly higher in stages 1, 2, 3 and 4 in patients with PCa than in those with BPH (p ≤0.001). Conclusions before the detection of cancer cells, the first changes in the metabolism of cells prone to cancer are observed via laboratory or radiological methods. Therefore, by periodic measurement of MMP-9 gene expression levels, protein levels and enzymic activity with methods used in the current study, it may become possible to diagnose prostate cancer before the formation of cancer cells and their invasion to other tissues, and thus, take very effective steps in the process of prostate cancer treatment.*

Keywords: *Prostate Cancer, MMP-9, Tumor Cell Metastasis.*

INTRODUCTION

In advanced countries, prostate cancer is the second most common form of cancer after skin cancer and the second most fatal cancer in men after lung cancer. Based on the studies conducted in laboratories and on alive animals, it has been proposed that overexpression of MMP facilitates tumor progression; thus, MMP inhibitors have been proposed as cancer treatment factors. It has now become clear that MMPs can be protective and useful; thus, a better understanding of MMPs' performance can improve targeting strategies against their malicious effects. MMPs have been considered biological markers. The presence of MMP-9 in natural glands is associated with higher Gleason scores and earlier stages of prostate cancer (Trudel et al., 2010). In a single-variable analysis of 278 patients with cancer limited to the prostate gland, among MMP-2, -3, -7, -9, -13 and -19, higher levels of MMP-9 expression had a protective effect on the overall rate of survival (Reis et al., 2011).

MMP-9, or gelatinase B, is secreted in an inactive form. This enzyme plays a major role in tumor progression. Genetic diversity, such as single nucleotide polymorphisms in the regulatory zone of MMP gene, can affect this enzyme expression. Due to its triple structure of fibronectin, MMP-9 can bind and digest collagen, as the most critical membrane composition. In this regard, changes in this gene's expression can be important in the formation of cancer cells and their behavior changes. Increased plasma level of MMP-9 can be observed in some types of malignant tumors such as breast cancer, colon cancer, lung cancer, head and neck cancer, hepatocellular carcinoma (HCC) and gastric cancer (Scaggiante et al., 2012; Aalinkeel et al., 2011). Approximately, 1.94% of prostate cancer cells express MMP-9 in the cytosol, and intracellular MMP-9 expression cannot be directly diagnosed by Gleason scores. The difference in MMP-9 expression may be partially explained by the difference in the degree of invasion of tumor specimens used in studies or by the sensitivity of diagnostic methods. MMP-9, obtained from tumor cells and tumor medium, plays an important role in the process of metastasis. In an experiment on immunodeficient mice, the MMP-9 host was significantly affected by prostate gland epithelium growth caused by transplanted osteolytic/osteogenic cells to calvaria (Aalinkeel et al., 2011; Gupta et al., 2013). It is imagined that changes in MMP-9 expression on a cellular level and forms of its secretion promote prostate cancer growth, and increase the rates of metastasis and angiogenesis (Xu et al., 2010; Xu et al., 2008). Tumor-stromal interactions can regulate MMP-9 expressions and their performance in prostate cancer (Inoue et al., 2000; Wang et al., 2011). In a co-culture of prostate cancer and stromal cells in a laboratory condition, pro-MMP-9 expression in prostate cancer cells and TIMPS expression in stromal cells increased (Johnson et al., 2010). Studies have shown that PDEF suppresses MMP-9 mRNA level and leads to a colonic reduction, cell migration and cellular invasion to prostate cancer cells (Lateef et al., 2013, Miyamoto et al., 2005). Androgens have a negative effect on MMP-9 expression; they significantly decrease MMP-9 secretion and activity in Androgen Receptor positive (AR+) prostate cancer cells (Dong et al., 2001). MMP-9 expression has been considered a factor that increases invasion and metastasis of tumor cells. PN-1 is a subset of MMP-9, which connects PN-1 destruction by MMP-9 to invasion regulation. Urokinase plasminogen activator (uPA) is inhibited by PN-1. Via PN-1 decomposition, MMP-9 boosts uPA activity in PC3-ML environment. PN-1 is separated from tumor cells by MMP-9. uPA is a PN-1 inhibitor. Increased PN-1 expression reduces uPA activity, MMP-9 increases PN-1 level and inhibits uPA activity, the effect of MMP-9 on uPA activity must be indirect, MMP-9 can be effective in PN-1 destruction (Xu et al., 2010). Sissak et al. (2003) observed that immunohistochemical on cellular proliferative activity, Ki-67 protein, MMP-9 matrix solution and MMP-1 inhibitor in cases of BPH and Adenocarcinoma (AC) with different Gleason scores significantly decreased MMP-9 and TIMP-1 levels in AC (Yousef et al., 2014). Yusef et al. (2014) showed that MMP-9 differential expression, indicating the degree of cell differentiation in breast cancer cells, is significantly associated with a variety of breast cancers (Rydlova et al., 2008). Prisciniks et al. (2007) found a relationship between tumor MMP-9 expression and histological type (Adenocarcinoma Mucosum) of pancreatic carcinoma (Safranek et al., 2007; Pryczynicz et al., 2007). Therefore, considering the prevalence of prostate cancer and the low sensitivity and specificity of PSA, as the most important laboratory biomarker for the diagnosis of prostate cancer, the present study was conducted to compare MMP-9 gene expression between patients with PCa and those with BPH, so that the disease can be diagnosed in a non-invasive, rapid and timely manner and the treatment process can become smoother.

Methodology

Sampling

To collect the study's samples, 63 patients with BPH (as the control group) and 30 with PCa (as the experimental group) were selected from among those referring to Shahada-ye-Tajrish Hospital.

RNA Extraction

The purity of mRNA extracted via absorption ratio, at a wavelength of 260-280 nm and in the range of 1.9-2 indicates non-contamination of the samples. Agarose gel is used to isolate and purify 0.2-5 kb-sized nucleotide fragments. With a specific agarose percentage, larger fragments will be better separated by TAE buffer, while the smaller ones will be better separated by TBE buffer. TAE buffer is more appropriate than TBE buffer in plasmid DNA analysis, while TBE buffer has a better buffering capacity in electrophoretic cases.

In the current study, the Real-time PCR technique was used to determine gene expression. Total RNA, obtained after measuring its concentration and assuring its quality, was used for cDNA synthesis. Kia Gene Company's kit was used to synthesize cDNA. The kit used the M-MLV reverse transcriptase. The primer used was called Oligo-(dT), a piece with 12-18 nucleotides attached to the PolyA tail in mammalian mRNAs; Oligo-(dT) can be used as a general primer for the production of cDNA. The AlleleID software was used to design the primer. The nucleotide sequences of the designed primers for MMP-9 and β-actin are presented in table (1). Based on the designed primers, the size of PCR products for MMP-9 and β-actin were respectively … and … bp.

Table 1. The sequence of primers designed for PCR of MMP-7, MMP-9, and β-actin genes

Size of PCR Product	Primer Sequence	Primer Name
Bp	5´- ATTGGCAATGAGCGGTTC-3´	β-actin forward
	5´-CAGCACTGTGTTGGCATAC-3.´	β-actin reverse
Bp	5´-CGTGACCTATGACATCCTG-3.´	MMP9 forward
	5´-TTCCTCCAGAACAGAATACC-3´	MMP9 reverse

Formation of Polyacrylamide Gel

In this study, the concentration was 10%, and the Polyacrylamide gel was provided as follows:

Table 2. Substances needed for gel preparation

Made solutions	10%
Acrylamide and Acrylamide Base	10
Separating Gel Buffer	5.2
Distilled Water	7.2
After mixing the mentioned substances, the solution was dehydrated for one minute with a vacuum pump, and then the following solutions were added to it.	
0.2 SDS	10%
TEMED	0.0.1
Ammonium Persulfate	0.1
TOTAL VOLUME	20

All results were provided as Mean ±Standard Deviation (SD). Using the SPSS software, between-group differences were analyzed via one-way ANOVA and Tukey's multiple comparison tests ($p \leq 0.05$).

Results

In the electrophoresis of agarose of RNA samples extracted from prostate cancer and BPH cells, only two rRNA-related bands were identified. Observing the two bands of 18S rRNA and 28S rRNA specified that the total RNA molecule, including mRNA molecules, had appropriate uniformity and quality. The Real-time reaction was performed with four dilutions of 1, 1.2, 1.4, 1.8 cDNA related to MMP-9. As can be seen in Figure (1), the sigmoidal reactions had basic phase, power amplification phase, and final stopping phase, indicating

the correctness of the reactions. As can be observed, the higher the cDNA concentration was, the sooner the related curve entered into its proliferation phase.
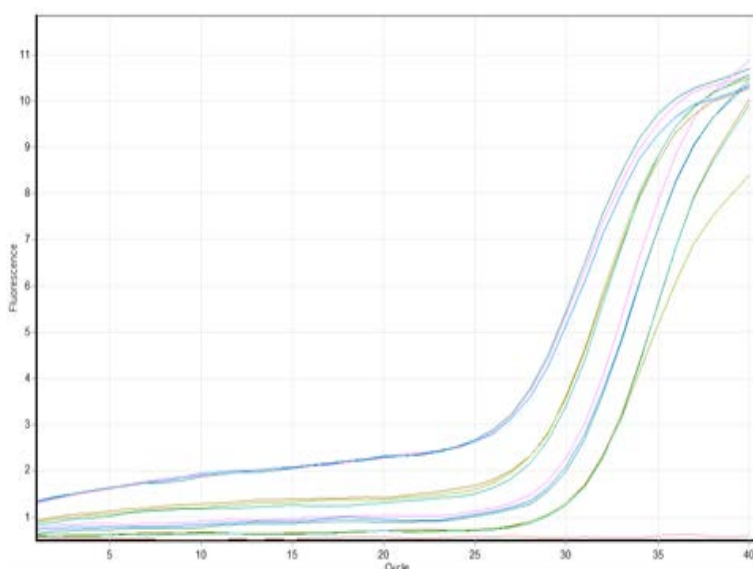

Figure 1. MMP-9 gene-related proliferation curve

To assure the accuracy and specificity of the reaction, fluorescence levels were measured at temperatures between 60 and 95 degrees. As can be seen in Figure (2), only one specific peak existed in the melting curve of the ß-Actin gene, indicating the specificity of the reaction.
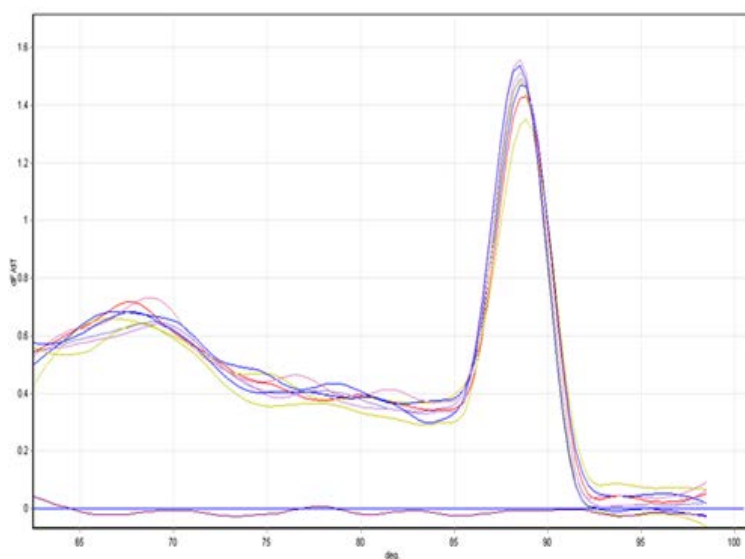

Figure 2. ß-Actin gene-related melting curve

To assure the accuracy and specificity of the reaction, fluorescence levels were measured at temperatures between 60 and 95 degrees. As can be seen in Figure (3), only one specific peak existed in the melting curve of the MMP-9 gene, indicating the specificity of the reaction. In other words, all nucleotide fragments in the reaction tube had identical sequence, length, and thus, melting point. In the case of experimental errors, fragments with different sequences and lengths and several peaks would be observed.
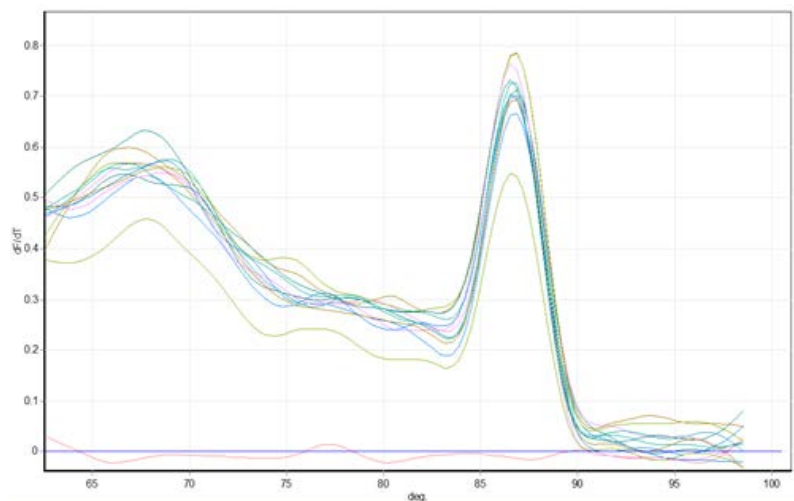
Figure 3. MMP-9 gene-related melting curve

The mRNA expressions of MMP-9 gene in prostate cancer tissue samples were significantly higher than in BPH tissue samples (p ≤0.001*) (Figure 4).
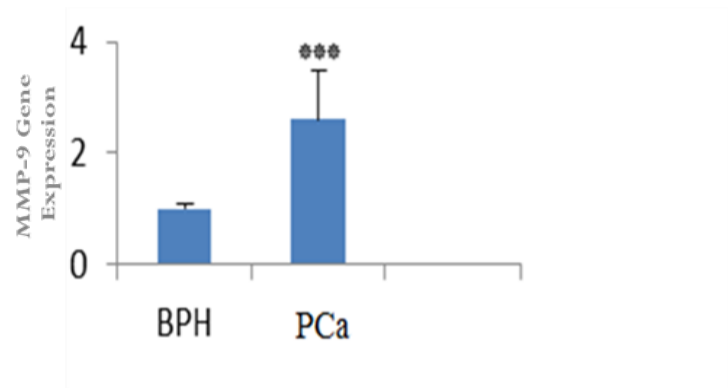

Figure 4. the comparison of MMP-9 expression between PCa and BPH patients

MMP-9 gene expressions of patients at stages 1, 2, 3, and 4 of prostate cancer were also compared with those of BPH patients (Figure 5). The results indicated that MMP-9 expression was significantly higher in PCa patients at every stage of prostate cancer compared to patients with BPH.
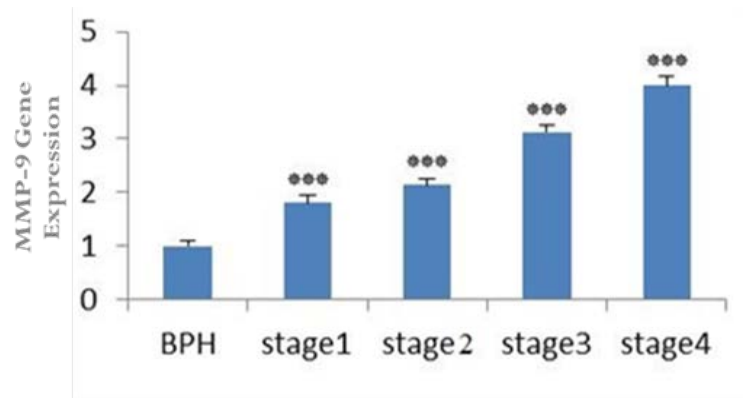

Figure 5. MMP-9 gene expression in PCa patients at different stages of the disease compared to that of the BPH patients
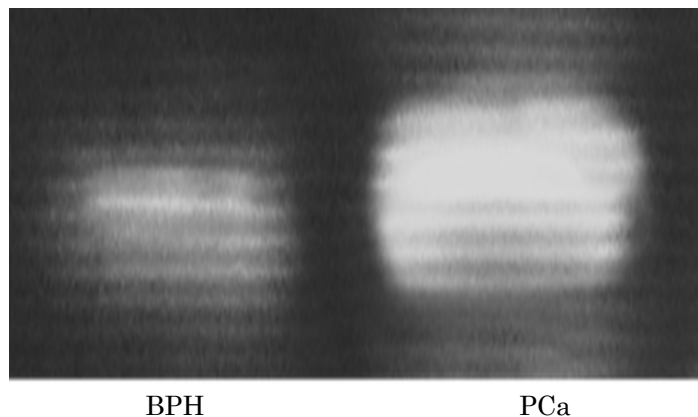
BPH   PCa

Figure 6. MMP-9 Zymography

As shown in Figure (6), the Zymography gel transparency in MMP-9 enzyme was more in the PCa group than in the BPH group, indicating that MMP-9 concentration and activity increased in prostate cancer sample tissues.

Discussion

In this study, MMP-9 gene expression was significantly higher in patients with prostate cancer than those with BPH (p ≤0.001*). Moreover, MMP-9 expression was also higher in patients at different stages of prostate cancer than those with BPH (p ≤0.001*). The results of MMP-9 gene expression analysis were confirmed using the Zymography technique. In many studies, a solid, but indirect relationship has been observed between MMP-9 invasion and tumor cell metastasis. Recently, two research teams have been able to establish a direct relationship between the concentration of this enzyme and tumor metastasis by using molecular genetic techniques and manipulating MMP-9 levels at cancer cells levels (Lakka et al., 2002; Lakka et al., 2002). In these studies, after transferring human cDNA to glial tumor cells, a three-fold increase in MMP-9 concentration and a 52% increase in invasion from the base membrane width were observed. By using the stable MMP-9 antisense oligonucleotide in glioblastoma cancer cells, Kondraganti et al. (2000) showed that protein concentrations and mRNA levels were lower in those cells. Those results confirmed the results of this study concerning that MMP-9 enzyme concentration was higher in prostate cancer tumor cells than in BPH cells. Furthermore, increased MMP-9 expression can catalyze the process of tissue renovation via inducing changes in vessel formation and activating receptors and vessel forming factors in prostate cancer cells (Aalinkeel et al., 2011).

Conclusions

The results of this study confirmed that MMP-9 expressions in samples collected from patients with prostate cancer were significantly higher than those collected from patients with BPH. Moreover, MMP-9 enzyme was higher in patients at different stages of prostate cancer compared to those with BPH. It seems hat MMP-9 increases the rate of cancer cell immigration and their invasiveness by increasing blood vessels via proteolytic destruction of collagen IV at their base membrane. Increasing MMP-9 expression level is a very potent factor in the metastasis process. This is most probably related to its role in the renovation and recovery of the extracellular matrix, and thus, the release of ECM or membrane-anchored growth factors. Therefore, by periodically measuring MMP-9 gene expression levels, protein levels and enzymic activity with methods used in the current study, it may become possible to diagnose prostate cancer before the formation of cancer cells and their invasion to other tissues, and thus, take very practical steps in the process of prostate cancer treatment.

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