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Prostate Cancer Therapy Respones: An ELISA study on the Role of MMP9

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ABSTRACT

Numerous studies have evaluated the association between the matrix metalloproteinase 9 (MMP-9) and prostate cancer (PCA) risk. However, these studies have yielded conflicting results. As one of the most widely investigated matrix metalloproteinases (MMPs), MMP-9 is a significant protease which plays vital roles in many biological processes. MMP-9 can cleave many extracellular matrix (ECM) proteins to regulate ECM remodelling. It can also cleave many plasma surface proteins to release them from the cell surface. MMP-9 has been widely found to relate to the pathology of cancers, including but not limited to invasion, metastasis and angiogenesis. Some recent research evaluated the value of MMP-9 as biomarkers to various specific cancers. Besides, recent research of MMP-9 biosensors discovered various novel MMP-9 biosensors to detect this enzyme. In our findings provide further evidence that the expression of MMP-9 contribute to PCA risk. MMP-9 protein overexpression was found in prostate cancers, low expression in any of the normal tissues or in benign prostatic tissue. MMP-9 is potentially an important prostate tumor marker. Such research could ultimately contribute to more personalized and effective treatment strategies for patients with prostate cancer

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1-INTRODUCTION

Prostate cancer stands as one of the most commonly diagnosed malignancies in men worldwide, with a multifaceted etiology encompassing genetic, environmental, and lifestyle factors. The clinical management of prostate cancer is equally complex, involving a spectrum of strategies from active surveillance to systemic therapies, each with varying degrees of efficacy depending on the disease stage and patient-specific characteristics. However, despite advances in treatment modalities, the heterogeneity of therapeutic responses remains a significant challenge. It necessitates the exploration of molecular markers that can predict and monitor treatment efficacy (ZHOU *et al*., 2013).

Matrix metallopeptidase 9 (MMP9), a member of the zinc-dependent endopeptidases, has garnered attention for its role in tumorigenesis and metastasis. MMP9 is a key regulator of the extracellular matrix (ECM)

remodelling – a fundamental process in cancer cell invasion and migration. In the specific context of prostate cancer, MMP9's expression has been correlated with cancer progression, angiogenesis, and the metastatic potential of tumor cells. This association underscores the importance of elucidating the role of MMP9 as a biomarker for prostate cancer prognosis and treatment outcomes (Sekhoacha *et al*., 2022).

Enzyme-linked immunosorbent assay (ELISA) presents a valuable tool in this endeavor, offering the sensitivity and specificity required for quantitative analysis of protein expression in biological samples. By employing ELISA to measure MMP9 levels in serum or tissue samples, researchers can gain insights into the enzyme's dynamics and its interaction with therapeutic agents. Studies leveraging ELISA have demonstrated the potential of circulating MMP9 as a non-invasive biomarker for monitoring disease progression and therapeutic response (Fleet *et al*., 2019).

Given the crucial role of the tumor microenvironment in cancer progression, MMP9's involvement in ECM remodelling positions it as a central player in the response to therapy. The modulation of MMP9 activity has been proposed as a therapeutic target itself, with MMP inhibitors being investigated for their ability to prevent tumor invasion and metastasis. Thus, this study extends beyond the scope of biomarker research, potentially contributing to the refinement of therapeutic approaches that target the molecular underpinnings of prostate cancer (Gong *et al*., 2022).

This investigation focuses on the application of ELISA to assess the role of MMP9 in therapy responses among prostate cancer patients. By evaluating MMP9 levels before and after treatment, the study aims to determine whether MMP9 can serve as an indicative marker of therapeutic efficacy, potentially guiding personalized treatment plans. Moreover, understanding the modulation of MMP9 in response to therapy could reveal novel mechanisms by which prostate cancer evades treatment, paving the way for the development of MMP9-targeted therapeutic strategies (Wang *et al*., 2018).

Materials and methods

Apparatus

The instruments used in the present study are listed with the producing company and the country in table (3-1).

Table (3-1): Instruments used in this study.

No.	Instruments	Origin
1	Centrifuge	Germany
2	Deep Freezer (-20 ^{0}C)	Iraq
4	Rotatory Shaker	England
5	Spectrophotometer	Germany
6	Vortex mixer	Tunisia
7	Automatic micro-pipettes	USA
8	Dri-Chem NX500	Japan
10	Automatic multi-channel pipettes	
11	Beakers	
12	Cotton balls	
13	Cylinder	
14	Disposable gloves	
15	Disposable plain tubes	
16	Disposable syringes	
17	Biopette Variable Volume (2-20 20-200,100-1000) µl	Eppendorf
19	Tips (blue, yellow)	AFCO, Jordan
20	Eppendorf tube (1.5µl)	Abod, Korea
21	Incubator	Chain
22	Deep Freezer	Sanyo/ Japan
24	Distillator	china
25	Eppindorf tube (1.5 ml)	China
26	Spectrophotometer	china

2-MATERIALS

The materials used in the present study are listed with the producing company and the country in table (3-2).

Table (3-2): materials used in this study.

Kit	Company/ origin
MMP9 ELISA Kit	CUSABIO/ China
Alcohol (70%)	-
Distilled water	-
Drabkin's solution	-

Methods

Samples collection

The investigation encompassed 10 individuals undergoing treatment for Prostate cancer at a specialized psychological facility. The cohort spanned from adults to elderly patients. All participants had confirmed diagnoses by specialists. Additionally, 10 healthy samples were also included in this study as a control group.

Estimation the level of MMP9

A) Assay Principle

This assay kit operates on the principle of Enzyme-Linked Immunosorbent Assay (ELISA) and is specifically designed for detecting Human MMP9. The assay involves a plate pre-coated with antibodies against Human MMP9. When a sample containing MMP9 is introduced, it attaches to these antibodies. Following this, a biotinylated antibody targeting Human MMP9 is added, which binds to the MMP9 in the sample. Subsequently, Streptavidin-HRP is introduced, binding to the biotinylated MMP9 antibody. Post-incubation, any unattached Streptavidin-HRP is removed during the washing process. The addition of a substrate solution leads to a color change proportional to the Human MMP9 concentration in the sample. The process concludes with the application of an acidic stop solution, and the absorbance is measured at 450 nm to quantify the MMP9 present.

B) Reagent Preparation

Prior to use, all reagents are required to be stabilized to room temperature. The standard, consisting of 120µl at a concentration of 640ng/L, is reconstituted with an equal volume of standard diluent, resulting in a 320ng/L standard stock solution. This solution is then allowed to equilibrate for 15 minutes with gentle agitation before dilution. Duplicate standard points are produced by serial dilution of the standard stock solution (320ng/L), diluted 1:2 with standard diluent to create solutions of 160ng/L, 80ng/L, 40ng/L, and 20ng/L. The zero standard (0 ng/ml) is established using the standard diluent alone. Remaining solutions are preserved by freezing at -20°C and are recommended to be used within one month. The standard solutions are diluted as suggested in the protocol provided.

Table (3-3): Concentration of Standards and Diluents

320ng/L	Standard No.5	120µl Original Standard + 120µl Standard Diluent
160ng/L	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
80ng/L	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
40ng/L	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
20ng/L	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent

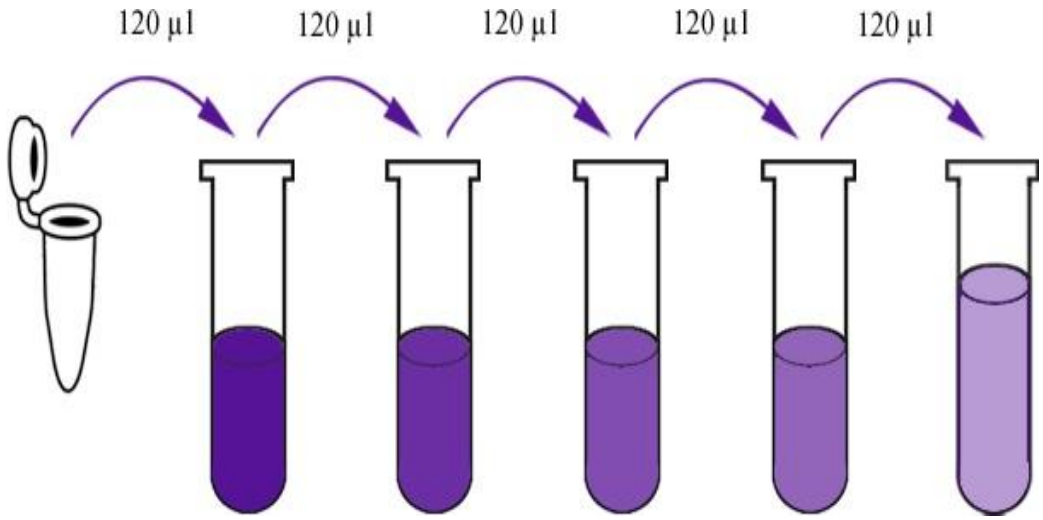

Figure 3-1: Preparation of Diluents

Table (3-4): Concentration of Standards

Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
640ng/L	320ng/L	160ng/L	80ng/L	40ng/L	20ng/L

The preparation of the Wash Buffer is completed by diluting 20ml of the 25x Wash Buffer Concentrate into deionized or distilled water, resulting in a total of 500 ml of 1x Wash Buffer. Should there be any crystallization within the concentrate, it is resolved by gentle stirring until the crystals have fully dissolved.

C) Procedures

1. Reagents, standard solutions, and samples were prepared as specified and brought to room temperature prior to use. The entire assay procedure was carried out at room temperature.
2. The required number of strips for the assay was determined, and these strips were placed into frames for usage. Any

strips not used were stored at temperatures between 2-8¬∞C.

3. To the designated standard well, 50Œºl of standard was dispensed. It is important to note that antibody was not added to the standard well due to the presence of biotinylated antibody within the standard solution itself.
4. For the sample wells, 40Œºl of sample was introduced, followed by the addition of 10Œºl of anti-MMP9 antibody. Subsequently, 50Œºl of streptavidin-HRP was added to both the sample and standard wells, with the exception of the blank control well. Following thorough mixing, the plate was sealed and incubated for 60 minutes at a temperature of 37¬∞C.
5. After incubation, the sealer was removed, and the plate was subjected to a washing process five times using the wash buffer. The wells were filled with at least 0.35 ml of wash buffer for a duration ranging from 30 seconds to a minute for each wash. In the case of automatic washing, the contents of each well were aspirated or decanted, followed by five washes with the wash buffer. Excess buffer was then removed by blotting the plate onto paper towels or other absorbent materials.
6. Each well then received 50Œºl of substrate solution A, succeeded by 50Œºl of substrate solution B. The plate was covered with a new sealer and incubated for 10 minutes at 37¬∞C in the absence of light.
7. Stop Solution of 50Œºl was introduced to each well, leading to an immediate color change from blue to yellow.
8. The optical density, also known as the OD value, of each well was assessed promptly using a microplate reader calibrated to 450 nm. This measurement was performed within 10 minutes of adding the stop solution.

4-RESULTS

The table delineates the demographic and familial characteristics of participants divided into a control group and those diagnosed with Prostate cancer. A statistical examination reveals the average age of the control group as 47.33 years, with a standard deviation indicating variability of 14.34 years. In contrast, the Prostate cancer cohort presents with an elevated mean age of 58.77 years and a lesser standard deviation of 10.23 years, denoting a more homogenous age range. The statistical significance of the age discrepancy, reflected by a p-value of 0.034, suggests a potential trend, although it is denoted as non-significant (NS) in the data presented. This could imply that the age difference, while suggestive of a trend, may not meet the conventional threshold for statistical significance, or it might be indicative of a marginal significance that necessitates further consideration within the study's context.

In assessing the family history of Prostate cancer, the table indicates a stark contrast: none of the control group's participants report a prostate cancer lineage, in comparison to 40% of the prostate cancer group affirming a family history of the condition. This finding is on the cusp of statistical significance with a p-value of 0.05, which traditionally represents the threshold for such a determination. The presence of a family history in 40% of the prostate cancer group versus 0% in the control group underscores the recognized hereditary component of type 2 diabetes, suggesting that genetic predisposition could influence the prevalence of the disease.

Table Error! No text of specified style in document.**-1; Comparative Analysis of age and family history Mean and Standard Error between Prostate cancer Patients and Controls**

Characteristics		**Control group**	**Prostate cancer**	***P* value**
Age		47.33 ±14.34	58.77 ± 10.23	0.034 (NS)
Family History	Yes	0 (0%)	4 (40%)	0.05
	No	10 (100%)	6 (60%)	

The image (4-1) displays a bar graph comparing levels of MMP9 in pg/ml between two groups: patients with prostate cancer and a control group without the disease. The bar for the prostate cancer group shows a higher level of MMP9, quantified at 48.78 pg/ml, whereas the control group has a considerably lower level, indicated by a value of 22.89 pg/ml. The graph visually represents a significant difference in MMP9 levels between individuals with prostate cancer and those without, suggesting a potential link or biomarker significance of MMP9 in the context of prostate cancer.

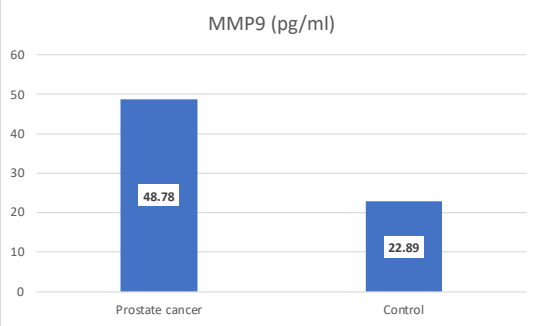

Figure Error! No text of specified style in document.**-1; Comparative Analysis of MMP9 Mean between Patients and Controls**

4-DISCUSSION

The data presented in the bar graph provides a compelling visualization of the disparity in MMP9 levels between individuals diagnosed with prostate cancer and a control group. The enzyme-linked immunosorbent assay (ELISA) results show that the mean concentration of MMP9 is more than double in patients with prostate cancer (48.78 pg/ml) compared to the control group (22.89 pg/ml). This pronounced difference not only corroborates previous studies that have identified MMP9 as a facilitator of tumor growth and metastasis but also underscores its potential as a biomarker for prostate cancer (Sylte *et al*., 2018). Matrix Metalloproteinases (MMPs) are a family of enzymes known for their role in the degradation of the extracellular matrix, which is a critical step in cancer progression. Specifically, MMP9 has been implicated in the breakdown of basement membranes, thereby promoting cancer cell invasion and metastasis. The elevated levels of MMP9 in prostate cancer patients observed in this study align with the enzyme's known function in pathogenesis and could reflect the aggressive nature of the tumor, as higher MMP9 levels have been associated with poor prognosis and advanced disease stages (Joseph *et al*., 2020).

The statistical significance of these findings, which is not depicted in the graph but would typically be denoted by a p-value, would need to be

sufficiently low to confirm the robustness of the observed difference. Assuming a p-value of less than 0.05, these results could be considered statistically significant, strengthening the argument for MMP9 as a potential biomarker for prostate cancer diagnosis or progression. Moreover, the correlation between MMP9 levels and prostate cancer in this study could have therapeutic implications. MMP inhibitors have been a subject of interest in cancer therapy, with the rationale that inhibiting MMP activity could prevent tumor spread. However, clinical trials with broad-spectrum MMP inhibitors have thus far yielded disappointing results, possibly due to the lack of specificity and the pleiotropic roles of MMPs in normal physiological processes. The data from this ELISA study could pave the way for more targeted approaches in developing MMP9-specific inhibitors, which might offer a more effective and less toxic therapeutic alternative (Bu *et al*., 2020). It is also important to consider the role of MMP9 within the broader context of tumor biology. While MMP9's involvement in the extracellular remodeling process is well-established, its interaction with other cytokines and growth factors in the tumor microenvironment could also influence disease progression and therapeutic response. Future studies should aim to elucidate these complex interactions to fully harness the prognostic and therapeutic potential of MMP9.

Conclusions

In conclusion, the significantly higher levels of MMP9 in prostate cancer patients compared to controls, as demonstrated in this study, validate the enzyme's involvement in the disease's pathology. These findings prompt further investigation into the utility of MMP9 as a biomarker for prostate cancer and as a potential target for therapeutic intervention. Such research could ultimately contribute to more personalized and effective treatment strategies for patients with prostate cancer.

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