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Original Article

Estimation of Matrix Metalloproteinases and their Tissue Inhibitors in Urine to Ascertain Noninvasive tools for Detecting Prostate Cancer

Authors
Sudipta Banerjee[1], Jayanta K Rout[2], Srabani Biswas[3], Avishek Sen Gupta[1], Saumitra Ghosh[1], Sangita Biswas[1]
[1]Dept. of Biochemistry, R G Kar Medical College, Kolkata-004, West Bengal, India
[2]Assistant Professor, Dept. of Biochemistry, R G Kar Medical College, Kolkata-004, West Bengal, India
[3]Professor, Dept. of Biochemistry, R G Kar Medical College, Kolkata-004, West Bengal, India
Corresponding Author
Dr Jayanta Kumar Rout
Assistant Professor, Dept. of Biochemistry, 4th Floor, Academic Building, R G Kar Medical College, 1, Kshudiram Bose Sarani, Kolkata: 700004 West Bengal, India
Email: *drsudipta1973@gmail.com*, Contact no:+919434978759

Abstract
Prostatic biopsy, though the gold standard investigation for prostate malignancy, often leads to unnecessary investigation with several complications. Our aim was to find out the cut off values, sensitivity, specificity and predictive values of urinary matrix metalloproteinases, their tissue inhibitors and free PSA to ascertain their suggestive role as noninvasive diagnostic tool for prostate cancer. Early morning urine samples of 40 clinically suggestive patients were collected and ELISA was done for MMP 7, 13, TIMP 1, 2 and free PSA followed by TRUS guided prostate biopsy. Values of MMP 7 and MMP 13 were found to have significant difference in levels between benign and malignant conditions. The cut off values in urine were obtained as free PSA>2.5mg/l, MMP 7>1ng/ml, MMP 13->75ng/ml, TIMP 1>4000pg/ml and TIMP 2>100pg/ml. MMP 13 and MMP 7 showed high sensitivity, specificity and predictive values while the tissue inhibitors and free PSA showed moderate to low sensitivity and specificity. MMP 13 having the most significant beta coefficient (0.263) was found to be the most significant parameter studied for diagnosing prostate carcinoma. Thus in the present study among different urinary parameters we found that MMP 13 is the most diagnostic tool for detecting prostate cancer followed by MMP 7 with doubtful roles of TIMP 1,TIMP 2 and free PSA.
Keywords: *mmp7, mmp 13, timp 1, timp 2, free psa.*

Introduction

Prostatic carcinoma has been ranked as the fourth most common malignant neoplasm all over the world[(1)] Prostate specific antigen (PSA)>4ng/ml is highly suggestive of prostatic carcinoma. But it needs to be confirmed by prostatic biopsy.

It has been found that only about 25% of men who have a prostate biopsy due to elevated PSA level actually have prostate cancer[(2)]. Again 20% of clinically significant prostatic cancer patients may have PSA values within normal range. So there is high chance of either unnecessary biopsies,

overdiagnosis and over treatment[3] or late detection. The need of the day is therefore to find out a new set of biomarkers for detecting prostate carcinoma along with total serum PSA.

Christenson and co-workers (1993) found out that serum free/total PSA cutoff at 0.18 (18% of free/total PSA) improves the ability to distinguish between cancer and non-cancer patients specially within the dubious range of serum total PSA 4-10 ng/ml.

Also the role of matrix-metalloproteinases (MMPs), a group of extracellular proteinases, in tumor invasion and metastasis by facilitating extracellular matrix degradation has been evident in different types of malignancies. Raised levels of serum MMP-13[4] and higher expression of MMP 1, 2, 7, 9, 11 and TIMPs (tissue inhibitors of MMPs) in tissue arrays has been found in prostate cancer[5, 6, 7].

Thus in cases of raised MMPs, there is probability of spilling over of MMPs from blood into urine [8] and raised levels can be detected in urine in prostate cancer. However TIMPs being a bifunctional enzyme, its role in inhibiting MMPs needs further clarification.

Our objective was therefore to find out whether MMPs, TIMPs and free PSA in urine can act as new non-invasive markers for early detection of prostate cancer along with total serum PSA.

Materials and Methods

Study design: Our study was a hospital based observational case control study conducted in the departments of Biochemistry and Urology.

Study population: A group of 40 patients clinically presenting with complaints of prostatism at the Urology Outpatient Department and total serum PSA >4 ng/ml were selected as sample for our study.

The study followed the guidelines of the Helshinki declaration of 2009 [9] and was approved by the Institutional Ethics Committee. Written consent was taken from every participant.

Inclusion criteria: As described in the study population.

Exclusion criteria

Patients on antibiotic therapy due to urinary tract infection, other urological diseases, no catheterization or operative intervention on bladder or prostate, absence of any history of malignancy (renal cell carcinoma, melanoma, breast and colorectal carcinoma) or metastatic disease receiving adjuvant chemotherapy were excluded from this group.

Collection and storage of sample

Early morning urine samples were collected from 40 such patients for future estimation of free PSA, MMP7, MMP13, TIMP-1 and TIMP-2. Samples were thawed to room temperature before every assay and repeated thawing was avoided.

Estimation of test parameters

Estimation of all the parameters were done by ELISA (Enzyme linked immunosorbent assay) with commercially available Accu Bind ELISA reagent kit for urinary free PSA (Lot #:EIA23KIA6H581) and Ray Biotech ELISA reagent kit for human MMP7 (Lot#:1219140222), MMP13 (Lot#: 1219140175), TIMP 1 (Lot #: 1219140191) and TIMP-2 (Lot#:12140192). Standard curve was done for each assay following the norms of the manufacturers.

TRUS guided multicore prostate biopsy was done in each case and the biopsy reports were collected in follow up.

Results

Patients were divided into two groups –benign (B, N=12) and malignant (M,N=28) according to biopsy results and statistical calculations were performed. 95% confidence interval was considered as significant ($p<0.05$) for all statistical tests done using SPSS and Med Calc for Windows.

Mean and standard deviation were calculated for each of the parameters (Table 1). Paired ‘t’ test was done and significant difference in values in benign and malignant conditions were obtained in cases of urinary free PSA,MMP 7, MMP 13,TIMP 1 and TIMP 2(Table 2).

Since no confirmed cut off value was available for urine from other studies, the ROC curve was drawn and cut off values were selected for each parameter (Table 3 and table 4). True and false positives and negatives of each of the parameters were calculated separately and the sensitivity, specificity, positive and negative predictive values of each of the parameters were then obtained (Table 5).MMP-13 was found to have the highest sensitivity (89.29%) and specificity (66.67%) considering the cut off value: >75ng/ml with high positive and negative predictive values followed by MMP-7 (sensitivity 78.57% and specificity 45.45% considering the cut off value of >1ng/ml). TIMP-1 has moderate sensitivity and specificity at cut off value >4000pg/ml while free PSA and TIMP-2 have very low sensitivity and specificity at cut off values >2.5ng/ml and >100pg/ml respectively.

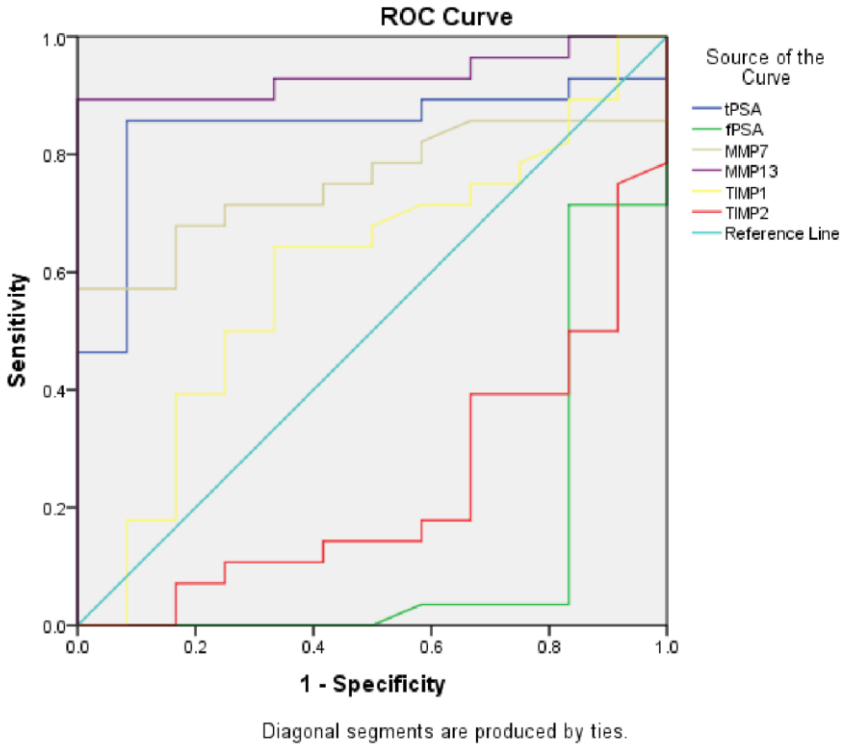
Beta coefficient of the parameters were calculated and was found to be most significant for MMP 13 (Table 6)

Table 1 showing mean and standard deviation of different parameters

Parameters	Group	No	Mean	Standard deviation
u PSA	Benign Malignant	12 28	9.044 2.869	6.1195 1.0252
MMP 7	Benign Malignant	12 28	1.658 5.528	1.1546 3.9817
MMP 13	Benign Malignant	12 28	67.075 102.505	9.8286 31.4405
TIMP 1	Benign	12	4381.833	2101.8800
	Malignant	28	5176.893	1932.2416
TIMP 2	Benign Malignant	12 28	223.442 98.988	211.7289 86.1726

Table 2 showing paired t test of different parameters in urine

Parameters	Equal variance assumed	Equal variance not assumed
uPSAt sig(2-tailed)	5.355 0.000	3.878 0.001
MMP 7 t Sig(2-tailed)	-4.308 0.000	-5.881 0.0000
MMP 13 t Sig(2-tailed)	-4.623 0.000	-6.368 0.000
TIMP 1 t Sig(2-tailed)	-1.781 0.081	-1.817 0.78
TIMP 2 t Sig(2tailed)	3.348 0.002	2.814 0.010



Area Under the Curve

Table 3 showing ROC curve of different parameters in urine

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
f PSA	.129	.079	.000	.000	.283
MMP7	.754	.075	.012	.608	.901
MMP13	.935	.040	.000	.856	1.000
TIMP1	.601	.099	.316	.407	.795
TIMP2	.234	.081	.008	.075	.392

Table 4 showing cut off values of different parameters obtained from ROC curve

Parametrs	Cut off values
MMP 13	75 ng/ml
MMP 7	1 ng/ml
TIMP 1	4000 pg/ml
TIMP 2	100 pg/ml
fPSA	2.5 ng/ml

Table 5 showing sensitivity, specificity, positive and negative predictive values of different parameters

Parameters	Sensitivity	Specificity	Positive predictive value	Negative predictive value
MMP 13	89.29	66.67	86.21	72.73
MMP 7	78.57	41.67	75.86	45.45
TIMP 1	67.86	50.00	76.00	40.00
TIMP 2	39.29	33.33	57.89	19.05
fPSA	58.33	16.67	56.33	16.56

Table 6 showing beta coefficient of the parameters

Model		Standardized Coefficients	t	Sig.
		Beta		
1	(Constant)		2.750	.009
	uPSA	(-) 0.478	-4.172	.000
	MMP7	0.130	1.156	.256
	MMP13	0.263	2.274	.029
	TIMP1	0.045	.433	.668
	TIMP2	(-) 0.375	-3.652	.001

Discussion

Prostatic carcinoma has been found to be one of the most common malignancies affecting the male population all over the world. To avoid unnecessary biopsies in patients with raised serum total PSA level>4ng/ml, a battery of parameters like free PSA, MMP 7, MMP 13, TIMP 1 and TIMP 2 were assayed in a noninvasive sample like urine in 40 suggestive patients for early detection of prostate cancer.

Hydrolysis of basic components of extracellular matrix by a group of 24 enzymes called matrix metalloproteinases which has been found to play a role in connective tissue invasion and distant metastasis. 77% and 50% of prostate tumors were found to focally express MMP 7 by in situ hybridization and western blotting[10,11]. Circulating MMP 7 level was found to be elevated in prostate cancer patients[12] and plasma MMP 13 always correlate to PSA values (1). Moss et al highlighted the possibility of detecting these markers in urine.

Our study also showed increased values of urinary MMP 7 and MMP 13 in malignant cases than in benign cases with significant difference in levels in cases and controls. The cut off values were calculated to be >1ng/ml for MMP 7 in urine with sensitivity 78.57% and specificity 41.67%. The sensitivity and specificity of MMP 13 in urine were found to be 89.29% and 66.67% respectively both having very good positive and negative predictive values.

Families of small extracellular proteins known as tissue inhibitors of metalloproteiases (TIMPs) often show decreased expression in consistent with the function of MMPs. However TIMPs were found to be paradoxically elevated in prostate cancer patients[14-16] probably due to its MMP independent action in the proliferation and inhibition of apoptosis[17-23] leading to promotion of tumor growth.

Our study also shows only slight difference in level of TIMP 1 in benign and malignant conditions with moderate sensitivity and specificity while TIMP 2 has been found to be higher in benign than in malignant conditions with low sensitivity and specificity.

In prostatic malignancy total bound PSA level has been found to be higher than free PSA as it escapes proteiolytic processing[24]. Also more free PSA isoforms has been found to be secreted from transitional zone (area of BPH) than from peripheral (malignant) zone. Our study also corroborates higher free PSA level in urine in benign than in malignant conditions. However the sensitivity, specificity and predictive values being low it cannot be considered alone as a significant diagnostic tool like total serum PSA.

Conclusion

Thus we can conclude from our study that values of different parameters like MMP 13 and MMP 7 in urine can help us to predict prostate carcinoma noninvasively in suggestive patients with serum total PSA >4 ng/ml and thus several unnecessary biopsies can be prevented. TIMP 1, TIMP 2 and free PSA in urine on the other hand have been found to have dubious role as diagnostic tools and needs further confirmation. Among all, MMP 13 can be considered as the most significant parameter to arrive at the diagnosis. However our study needs corroboration from further studies

over longer duration to arrive at a definite conclusion.

Compliance with Ethical Standards

Funding: NIL (As it was a dissertation of a post graduate trainee hence, question of funding is not applicable)

Disclosure of potential conflicts of interest: NIL

Research involving human participants and/or animals: Human participant were taken

Consent of participation: All the participants were informed about the details of the study and written consent was taken from each of them.

Ethical Consideration: Approved by the Institutional Ethics Committee

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