ORIGINAL RESEARCH PAPER

Oncology

URINARY PROSTATE-SPECIFIC ANTIGEN AND MICROSEMINOPROTEIN-BETA LEVELS IN MEN WITH AND WITHOUT PROSTATE CANCER: A PROSPECTIVE STUDY OF 60 CASES IN INDIAN POPULATION.

KEY WORDS: PSA, MSMB, DRE, PC.

Dr Shreenath Patil Department of Urology, A.J. Institute of Medical Sciences and Research Center, Mangalore, Karnataka, India.

Dr Akash Bande* Department of Urology, A.J. Institute of Medical Sciences and Research Center, Mangalore, Karnataka, India. *Corresponding Author

Dr Yogendra Chidrawar Department of Urology, A.J. Institute of Medical Sciences and Research Center, Mangalore, Karnataka, India.

ABSTRACT

Background: The role of urinary proteomics in the diagnosis of prostate cancer (PC) is undefined. Levels of urinary biomarkers such as prostate-specific antigen (PSA) and microseminoprotein-beta (MSMB) may differ between men with and without PC. We tested this hypothesis using urine samples before and after digital rectal examination (DRE) in men with an indication for prostate biopsy.

Methods: This prospective study was performed after all individuals provided informed consent for inclusion, men with elevated PSA or a nodule on DRE underwent a pre and post-DRE urine sample examination for urinary PSA and MSMB levels. Levels were compared between men who had PC diagnosed on biopsy (Group A) and those with a negative biopsy (Group B).

Results: A total of 60 patients were included in the study, 2 patients had very high serum PSA values (2315 and 1699 ng/ml) and were excluded from the analysis as outliers. 58 patients were included of whom 21 had PC (Group A) and 37 had no cancer (Group B) on biopsy. The median urine PSA (25.4 vs 22.3 mg/dl) and MSMB (1.9 vs. 1.2 mg/dl) were almost similar in both groups at baseline. However, Both urinary PSA and MSMB values rose significantly after DRE in Group B (median urinary PSA from 22.3 to 99.2 mg/dl; median urinary MSMB from 1.2 to 4.3 mg/dl) but not in Group A (median urinary PSA from 25.4 to 24.1 mg/dl; median urinary MSMB from 1.9 to 3.1 mg/dl)

Conclusions: Urinary PSA and MSMB rose significantly after DRE only in men without PC. Post-DRE urine PSA and MSMB can differentiate PC from benign pathology in men with an indication for prostate biopsy.

INTRODUCTION

Prostate cancer (PC) is the second most common cancer among men worldwide [1]. Measurements of prostate specific antigen (PSA) in blood are widely used to detect men at PC-risk and monitor men with PC. Due to low test-specificity at moderately elevated levels, indiscriminate use of PSA-testing results in large number of unnecessary biopsies and over-diagnosis with a consequent risk of overtreatment [2, 3]. Hence, there is an urgent need for additional biomarkers that can provide enhanced specificity for the early detection of aggressive, lethal forms of the disease.

Microseminoprotein-beta (MSMB) is one of the most highly secreted proteins from the prostate, and circulating levels have been shown to be positively correlated (r = approximately 0.2) with both levels of total and free prostate-specific antigen (PSA) [4]. In contrast with PSA, whereby risk of prostate cancer increases with higher PSA levels, MSMB levels measured in serum, urine, and prostate tissue have been shown to be statistically significantly lower in men with prostate cancer and even lower in men with aggressive disease [5,6]. The reproducible association of rs10993994 with prostate cancer risk and circulating MSMB levels implicates MSMB in the etiology of prostate cancer [7].

To determine the prospective relationship between circulating pre-diagnostic levels of MSMB and prostate cancer risk, we measured MSMB and PSA in men with and without prostate cancer.

METHODS

This prospective study was performed after all individuals provided informed consent for inclusion, total 60 patients included in this study. Men scheduled to undergo transrectal ultrasound (TRUS) - guided biopsy of the prostate for suspicion of PC due to either elevated PSA (>4 ng/mL) or a nodule on DRE were recruited. A sterile urine culture was confirmed before inclusion. All individuals provided a morning urine specimen followed by a digital rectal examination (DRE), including prostatic massage. Prostatic massage was performed using firm pressure, sufficient to depress prostate by about 1 cm, with three strokes for each lobe. Each stroke applied from the lateral to midline and from the base to the apex for each lobe [8]. Immediately after the DRE, urine was collected as the post-DRE sample. The pre-DRE urine collected was also used for a routine urine examination. The outcome measures included the estimation of pre-DRE and post-DRE urine samples for PSA, MSMB, and their ratio for the assessment of their discriminative ability for PC.

Both the pre-DRE and post-DRE samples were used for the estimation of urinary total PSA and MSMB protein level by ELISA. Collected urine was centrifuged at 1500 rpm for 10 min to sediment the bacterial contamination, crystals, cell debris, etc. One aliquot was kept for the determination of urine creatinine. The supernatant of the urine was filtered through pressure-based amicon filters. Initially, 100 ml of the urine was filtered through 30 kD amicon filters. The flow-through of this step was filtered again using 10 kD amicon filter. The filtration was carried out until 5 ml of the urine concentrate was left. The remnant urine was highly concentrated with protein in the range of 10–30 kD. Concentrates were aliquoted to 1 ml and stored at 80°C for long term storage. Patients with PC on biopsy were labeled as Group A and were staged for disease on the basis of the Gleason score of their prostate biopsy, and clinical staging was done according to the 8th American Joint Committee on Cancer version released in 2016.

Statistical analysis

The data are presented as mean (± standard deviation [SD]) or median (interquartile range [IQR]) as appropriate. Paired or unpaired t-test or Wilcoxon signed rank-sum test were used to compare quantitative variables while Chi-square/ Fisher's exact test was used for qualitative variables.

RESULTS

A total of 60 patients were included in the study. 2 patients had very high serum PSA values (2315 and 1699 ng/ml) and were

excluded from the analysis as outliers. Of 58 patients, 21 had cancer and were included in Group A while 37 with a negative biopsy were included in Group B. The mean age (± SD) was 68.1 (±6.3) years in group A and 64.8 (±5.6) in Group B. The median (IQR) serum PSA was 45.7 (1.23–679.2) ng/ml in Group A and 6.4 (0.01–32.8) ng/ml in Group B. The mean prostate volume (±SD) was 49.2 (±29.6) cc in Group A while 47.3 (±26.3) cc in Group B. 17 patients (80.9%) in Group A had abnormal DRE, while 16 patients (43.2%) of Group B had abnormal DRE findings. MRI was done in 18 patients (85.7%) in Group A and 16 patients (43.2%) in Group B. All patients underwent TRUS-guided biopsy of the prostate with at least 12 cores, with or without MRI fusion. 3 patients (8.1%) of Group A underwent TRUS-guided biopsy with MRI fusion while 8 patients (21.6%) underwent TRUS-guided biopsy with MRI fusion in Group B.

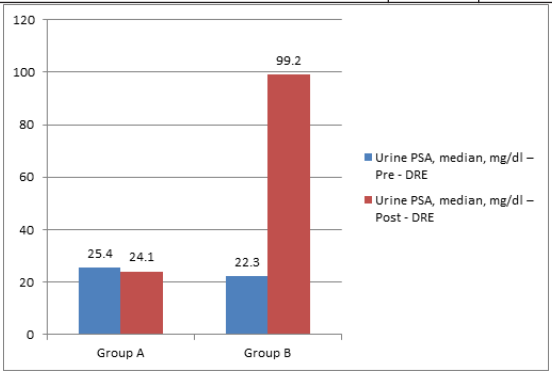
Table 1 : Multiple Parameters of Patients and Investigation done on them.

Parameters	Group A	Group B
Number of patients	21	37
Age (years), mean ± SD	68.1 ± 6.3	64.8 ± 5.6
Serum PSA, median (IQR), ng/ml	45.7 (1.23 – 679.2)	6.4 (0.01 – 32.8)
Abnormal digital rectal examination, n (%)	17 (80.9)	16 (43.2)
Prostate Volume (cc), mean ± SD	49.2 (±29.6)	47.3 (±26.3)
Patient undergoing multiparametric MRI, n (%)	18 (85.7%)	16 (43.2)
Patients undergoing MRI-TRUS fusion biopsy, n (%)	3 (8.1)	8 (21.6)

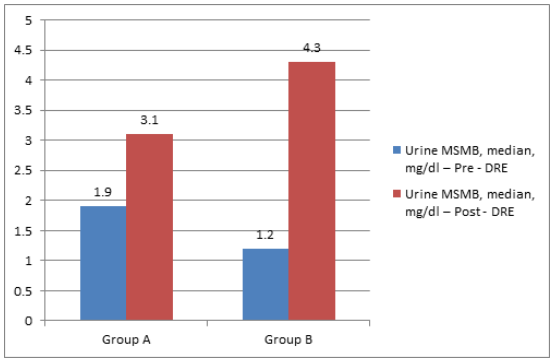
The expression of urine PSA and MSMB before and after DRE in the two groups is shown in Table 2. Baseline urinary PSA and MSMB were similar in both groups. Both urinary PSA and MSMB values rose significantly after DRE in Group B (median urinary PSA from 22.3 to 99.2 mg/dl [P = 0.001]; median urinary MSMB from 1.2 to 4.3 mg/dl [P = 0.001]) but not in Group A (median urinary PSA from 25.4 to 24.1 mg/dl; median urinary MSMB from 1.9 to 3.1 mg/dl). In between group comparisons, while pre-DRE urinary PSA values were almost similar in both groups, post-DRE values were significantly higher in Group B in comparison with Group A (P = 0.001). Urinary MSMB values showed a similar trend and were similar in the two groups before DRE but were significantly higher after DRE in Group B than in Group A (P = 0.001).

Table 2 : Urinary prostate-specific antigen and β-microseminoprotein before and after digital rectal examination

Parameters	Group A	Group B
Urine PSA, median, mg/dl – Pre - DRE	25.4	22.3
Urine PSA, median, mg/dl – Post - DRE	24.1	99.2
Urine MSMB, median, mg/dl – Pre - DRE	1.9	1.2
Urine MSMB, median, mg/dl – Post - DRE	3.1	4.3



Graph 1 : Urinary prostate-specific antigen before and after digital rectal examination



Graph 2: Urinary β-microseminoprotein before and after digital rectal examination

DISCUSSION

In our study we observed that although baseline urinary PSA and MSMB were almost similar with and without PC, both these parameters were significantly elevated post - DRE in men who did not have PC while the change was not significant among men with PC.

Our finding of higher post-DRE urinary PSA and MSMB as compared to pre-DRE samples in patients without PC are similar to those reported by Drake et al [9]. They characterized the constituents in post-prostatic massage urine sample using proteomic studies and reported the utility of these constituents as potential biomarkers and therapeutic targets in PC. They also reported low levels of urine PSA in post-DRE samples in patients with PC

The exact mechanism of this phenomenon is yet to be determined. It is possible that DRE causes liberation of PSA into the urine. In men without PC, cellular architecture within the prostate gland is maintained with intact cell membranes, ductal anatomy, and normal drainage of prostatic secretions into the urethra. Prostatic manipulation may stimulate secretion of proteins and other molecules/exosomes into the urethra through the intact ducts, causing a rise. However, in PC, there is cellular disarray with compression/stenosis and disruption of prostatic ducts with neovascularity and loss of cellular polarity with the release of secreted molecules into the blood circulation across the basement membrane leading to rising in serum levels of PSA. It is known that DRE results in increased serum PSA [10, 11] and thus, prostatic manipulation may not liberate any additional urinary PSA, explaining the rise in post-DRE PSA specifically in men without PC.

In benign prostate, MSMB regulates cell growth by promoting apoptosis while in malignancy; there is loss or decreased MSMB expression, leading to uncontrolled growth of cells. This difference becomes more prominent in post-DRE urine samples, and the rationale appears to be similar to that for urinary PSA. Prostatic massage liberates MSMB in men with normal glands (without PC), causing a rise while there is no additional release in men with PC, thus heightening the difference between the two groups. Whitaker et al. [12] studied urinary levels of MSMB in 215 patients without PC (normal/BPH) and 89 patients with PC. They reported a significant decrease in urinary MSMB in patients with PC than those without PC (P < 0.001), consistent with findings in prostatic tissue. They also reported urinary MSMB to be significantly better as compared to urinary PSA but not better than serum PSA for the diagnosis of PC on analysis of ROC curves [12]. Previous studies have reported higher levels of urinary MSMB in benign/normal prostatic tissue or serum than in tissue or serum from PC patients [12-14] which are almost similar to our study.

CONCLUSION

Urinary PSA and MSMB rose significantly after DRE in men

without PC but not in men with PC. Post DRE levels were significantly different between the two groups. These noninvasive urinary biomarkers may have a role in identifying patients more likely to have PC among men with an indication for prostate biopsy.

Acknowledgement: none
Conflict of interest: none
Funding: none

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 2015 Mar 1; 136
2. Schroder FH, Hugosson J, Roobol MJ, Tammela TL, Zappa M, Nelen V, et al. Screening and prostate cancer mortality: results of the European Randomised Study of Screening for Prostate Cancer (ERSPC) at 13 years of follow-up. Lancet 2014 Dec 6; 384(9959):2027–2035.
3. Vickers AJ, Sjoberg DD, Ulmert D, Vertosick E, Roobol MJ, Thompson I, et al. Empirical estimates of prostate cancer overdiagnosis by age and prostate-specific antigen. BMC Med 2014 Feb 11; 12:26- 7015-12-26.
4. Waters KM, Stram DO, Le Marchand L, et al. A common prostate cancer risk variant 5' of microseminoprotein-beta (MSMB) is a strong predictor of circulating beta-microseminoprotein (MSP) levels in multiple populations. Cancer Epidemiol Biomarkers Prev. 2010;19(10):2639–2646.
5. Nam RK, Reeves JR, Toi A, et al. A novel serum marker, total prostate secretory protein of 94 amino acids, improves prostate cancer detection and helps identify high grade cancers at diagnosis. J Urol. 2006;175(4): 1291–1297.
6. Whitaker HC, Kote-Jarai Z, Ross-Adams H, et al. The rs10993994 risk allele for prostate cancer results in clinically relevant changes in microseminoprotein-beta expression in tissue and urine. PLoS One. 2010;5(10): e13363.
7. Whitaker HC, Warren AY, Eeles R, et al. The potential value of microseminoprotein- beta as a prostate cancer biomarker and therapeutic target. Prostate. 2010;70(3):333–340.
8. Graves HC, Sensabaugh GF, Blake ET. Postcoital detection of a male-specific semen protein. Application to the investigation of rape. N Engl J Med 1985;312:338-43.
9. Drake RR, White KY, Fuller TW, Igwe E, Clements MA, Nyalwidhe JO, et al. Clinical collection and protein properties of expressed prostatic secretions as a source for biomarkers of prostatic disease. J Proteomics 2009;72:907-17.
10. Chybowski FM, Bergstralh EJ, Oesterling JE. The effect of digital rectal examination on the serum prostate specific antigen concentration: Results of a randomized study. J Urol 1992;148:83-6.
11. Coleen MA, Thomas JD, David SM, Anthony PP, Lana T, Jill W, et al. Effect of digital rectal examination on serum prostate-specific antigen in a primary care setting. The internal medicine clinic research consortium. Arch Intern Med 1995;155:389.
12. Whitaker HC, Kote-Jarai Z, Ross-Adams H, Warren AY, Burge J, George A, et al. The rs10993994 risk allele for prostate cancer results in clinically relevant changes in microseminoprotein-beta expression in tissue and urine. PLoS One 2010;5:e13363.
13. Iwakiri J, Granbois K, Wehner N, Graves HC, Stamey T. An analysis of urinary prostate specific antigen before and after radical prostatectomy: Evidence for secretion of prostate specific antigen by the periurethral glands. J Urol 1993;149:783-6.
14. Wa ter s KM, Stram DO, Le Ma rchand L , Klein RJ , Valtonen-Andre C, Peltola MT, et al. A common prostate cancer risk variant 5' of microseminoprotein-beta (MSMB) is a strong predictor of circulating beta-microseminoprotein (MSP) levels in multiple populations. Cancer Epidemiol Biomarkers Prev 2010;19:2639-46.