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Pathology

A STUDY TO CORRELATE HISTOPATHOLOGY, SERUM PSA LEVEL AND IMMUNOHISTOCHEMICAL EXPRESSION OF ER & PR IN PROSTATIC GROWTH

Dr. Prashant Bhardwaj	MBBS, MD, Assistant Professor, Department of Pathology, Rajshree Medical Research Institute, Fatehganj Paschimi, Bareilly-243501
Dr. Sumit Nandy	MBBS, MD, Assistant Professor, Department of Pathology, Calcutta National Medical College, Kolkata.
Dr. Keya Basu	MBBS, MD, Professor & HOD, Department of Pathology, KPC Medical College, Kolkata.
Dr. Manjari Kumari*	MBBS, MD, Assistant Professor, Department of Pharmacology, Rajshree Medical Research Institute, Fatehganj Paschimi, Bareilly-243501 *Corresponding Author
Dr. Dilip Karmakar	MBBS, MS, MCH, Professor & HOD, Department of Urology, Calcutta National Medical College, Kolkata.

ABSTRACT **Objectives-** Prostate is a fibromusculoglandular structure situated at the neck of urinary bladder. It enlarges due to benign hyperplasia of prostate (BHP), prostatic intraepithelial neoplasia (PIN) or adenocarcinoma. Enlargement of prostate is associated with raised serum level of prostate-specific antigen (PSA) and altered expression of estrogen receptor (ER) and progesterone receptor (PR). The aim of our study is to correlate the histopathology, PSA levels and altered expression of ER and PR by immunohistochemistry in different prostatic growth lesions.

Methodology- Patients diagnosed as having prostatic growth were enrolled and their serum PSA levels were noted. Histopathological examination and immunohistochemical analysis of prostatic tissues for ER and PR were carried out to find out correlation of different type of growth with serum PSA level and expression of ER and PR.

Results- A total 96 cases studied of them 61(63.54%) patients presented with BHP, 20(20.83%) patients with BHP with chronic prostatitis, 3 patients presented with metaplastic changes, 5 cases with of PIN and 6 patients presented with adenocarcinoma with different Gleason score. PR expression positivity in epithelial cells and stromal cells of BHP cases were 51(83.6%) and 53(86.88%) respectively. Patients presented with adenocarcinoma showed only 33.33 %(2cases) positivity in epithelial cells and 50% (3cases) positivity in stromal cells. Serum PSA level were significantly higher in adenocarcinoma patients as compare to BHP patients.

Conclusion- By observing these findings it can be suggested that and antiprogesterone therapy may be helpful in the treatment of prostatic adenocarcinoma.

KEYWORDS : Immunohistochemistry, Progesterone receptor, Estrogen receptors, Prostatic growth, Prostate Specific Antigen

INTRODUCTION

Prostate is a pyramid-shaped fibromusculoglandular organ lies below the urinary bladder and is located in front of rectum. The apex of prostate is directed downward and upward directed base in contact with urinary bladder and seminal vesicles. The weight of prostate is about 20 g in adults but size is variable according to age.[1] Anatomically prostate is divided into transition zone, central zone and peripheral zone.[2] These zones have different biological and histological characteristic.The benign prostatic hyperplasia (BPH) exclusively arise in transition zone while in peripheral zone most of the carcinomas and prostatic intraepithelial neoplasia (PIN) develop.[3]

Benign prostatic hyperplasia (BPH) is common in older male and generally accompanied by urinary symptoms like frequency, urgency, nocturia, dysurea etc. The prevalence of prostatic hyperplasia increases with age from 8% in men aged 31 to 40years, to 40 to 50 % in men aged 51 to 60years. Is exceeds to 80 % in men older than age 80years.[4,5]

The premalignant proliferative changes in prostatic glandular epithelium are of two types; first one is prostatic intraepithelial neoplasia (PIN) and second is atypical adenomatous hyperplasia (AAH). High-grade PIN is the most commonly observed premalignant lesion in adenocarcinoma of prostate while AAH shows a weaker association with carcinoma.[6] Carcinoma of prostate commonly occurs in elderly individuals. More than 75% of carcinoma cases occurring in men above 65 years of age. Prostatic cancer is the second most frequently diagnosed cancer in men worldwide and the fifth most common cancer overall.[7]

Estrogen receptor (ER) and Progesterone receptor (PR) are members of nuclear receptor family. Estrogen receptor (ER) having two isoforms ERα and ERβ. Estrogen receptors and estrogen are associated with tumourogenesis, tumour progression and tumour evolution of prostate.[8] Progesterone receptors are also play important role in development of carcinoma of prostate. Stromal PR has inhibitory effect on proliferation of both epithelial and stromal components.[9]

Small amount of prostate specific antigen (PSA) is secreted by prostatic epithelium into the secretory ducts. In different type of prostatic lesions serum PSA level is elevated. The level of serum PSA is much higher in carcinoma prostate as compare to other prostatic lesions like BHP and prostatitis. Diet alterations, medications and environmental factors also associated with raised serum PSA level.[10] Keeping these facts in our mind, our study objective was to correlate the histopathological findings of different prostatic growth lesions with PSA level in serum and expression of ER and PR by immunohistochemistry (IHC).

MATERIAL AND METHOD

The study was conducted in the Department of Pathology & department of Urology of Calcutta National Medical College & Hospital (C.N.M.C. & H), Kolkata, West Bengal. A total of 96 cases were collected which were diagnosed in Department of Urology as cases of prostatic growth. Among them, TURP specimens were received in maximum numbers 81(85%). TRUS biopsy and prostatectomy specimen were 9 and 6 respectively. Gross details of sent specimens along with clinical, radiological and serum PSA level of all patients were noted in predesigned case record form for each specimen. Haematoxylin and Eosin (H&E)-stained sections were prepared for routine histopathological evaluation to see nature of growth including Gleason scoring in cases of carcinoma. Poly-l-Lysine-coated slides were used for immunohistochemical staining for, ER and PR.

The study data collected in case record forms were transcribed onto a MS excel datasheet and analysed using SPSS (Statistical Package for Social Sciences, version 16.0.1 of IBM, USA) and GraphPad Prism Version 5.0 of GraphPad Software, USA statistical software, considering a 'p' value below 0.05 as significant.

RESULT AND ANALYSIS

We studied a total of 96 cases of them 61(63.54%) patients presented with BHP and 20(20.83%) patients had BHP with chronic prostatitis. 3 patients presented with metaplastic changes in prostate and one with prostatic infracts. Cases of high grade PIN and low grade PIN were 3(3.15%) and 2(2.08%) respectively. Patients presenting with adenocarcinoma with different Gleason score were 6(6.25%). **(Chart-1)**

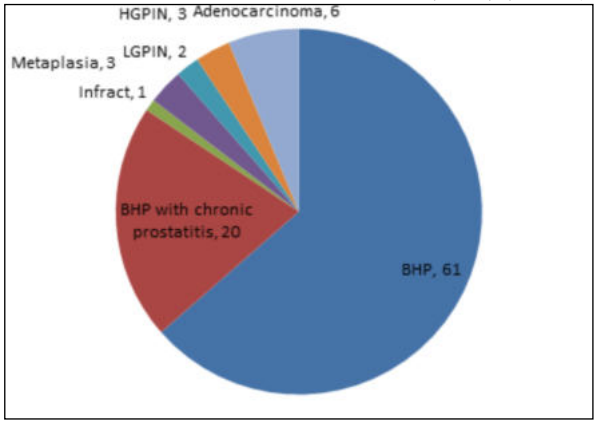
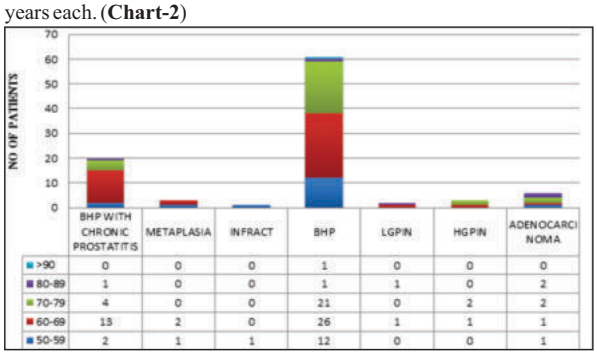


Chart 1: Frequency Of Different Prostatic Lesions

The number of patients of BHP and BHP with chronic prostatitis was maximum in age group of 60-69 years and that were 26(42.62%) and 13(65%) respectively. The mean age of BHP cases was 65.77± 7.68years and 49(80.32%) (CI-69.19 to 91.45%) patients were more than 60 year of age. In case of BHP with chronic prostatitis mean age were 66.5±6.9 years. Mean age of patients presented with PIN and adenocarcinoma was 69.4±7.33years and 71±11.24 years respectively. In case of adenocarcinoma, 50-59 and 60-69 years age group had one patient in each group and two patients in age group 70-79 and 80-89 years each. (**Chart-2**)



	BHP WITH CHRONIC PROSTATITIS	METAPLASIA	INFRACT	BHP	LGPIN	HGPIN	ADENOCARCINOMA
>90	0	0	0	1	0	0	0
80-89	1	0	0	1	1	0	2
70-79	4	0	0	21	0	2	2
60-69	13	2	0	26	1	1	1
50-59	2	1	1	12	0	0	1

Chart 2: Age Distribution Of Different Types Of Lesions

A total 54 (88.52%) BHP cases showed serum PSA level less than 4ng/ml and in remaining 7 cases the level of PSA laid in between 4ng/ml to 10ng/ml. None of BHP cases exceed the limit 10ng/ml and mean value of PSA was 3.6±1.2ng/ml. Serum PSA level in majority of BHP cases were found to < 4ng/ml as compared to chronic prostatitis with a highly significant *p* value of 0.0001. Similarly, there was statistically significant difference in serum PSA level between BHP and Adenocarcinoma cases with a *p* value of 0.004. (Test of significance used- Fischer exact test).

In case of chronic prostatitis 13(65%) patients had serum PSA level in between 4ng/ml to 10ng/ml while remaining 7 patients had serum PSA level below 4ng/ml. The mean PSA value was 4.57±0.81 ng/ml. This mild elevation of serum PSA level in chronic prostatitis is statistically significant with a *p* value of 0.0003.

A total 4 (80%) patients with PIN showed serum PSA level in between 4 to 10ng/ml and only one patient exceeded the limit of 10ng/ml. The mean PSA level in PIN cases was 8.6±1.06 ng/ml. Of all 6 patients with adenocarcinoma, 5 cases had serum PSA more then10 ng/ml and mean PSA is 102.2±58.62ng/ml. Only one case had PSA value less than 10ng/ml. **(Chart- 3)**

PR expression positivity in epithelial cells and stromal cells of BHP cases were 51(83.6%) and 53(86.88%) respectively**. (Figure-1,2)** In BHP with chronic prostatitis, PR positivity in epithelial cells and stromal cells was seen in 80% (16) and 85% (17) respectively. Patients with PIN showed 60% positivity for PR receptors by both epithelial and stromal cells. Patients presented with adenocarcinoma showed only 33.33 %(2cases) positivity in epithelial cells and 50 %(3cases) positivity in stromal cells. **(Chart- 4) (Figure 3, 4, 5, 6)** PR expression in epithelial cells in BHP cases was found to be is significantly higher than PR expression in tumour epithelial cells of adenocarcinoma with *p* value of 0.0149. BHP with chronic prostatitis cases showed approximately same results as in BHP. Of total 96 different types of cases the expression of ER was negative in all received samples.

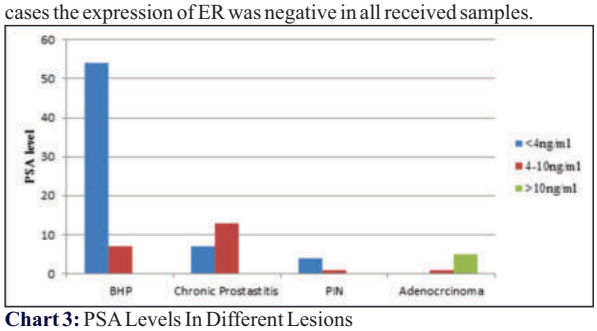
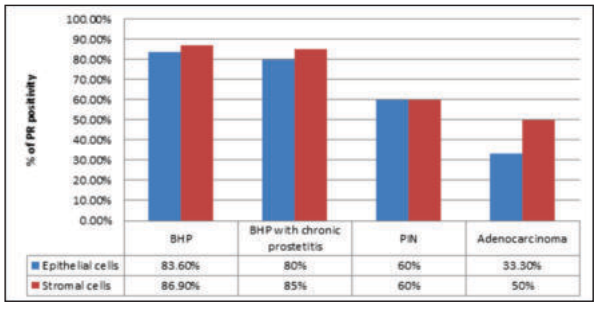


Chart 3: PSA Levels In Different Lesions



	BHP	BHP with chronic prostetitis	PIN	Adenocarcinoma
Epithelial cells	83.60%	80%	60%	33.30%
Stromal cells	86.90%	85%	60%	50%

Chart 4- PR Receptors Expression In Different Lesions

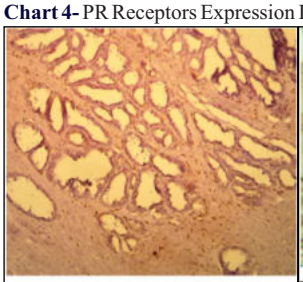


Figure 1- Section of BHP showing PR positivity in stromal cells (10 x)

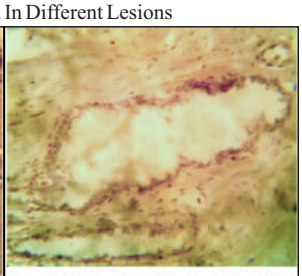


Figure 2- Section of BHP showing PR positivity in epithelial cells and stromal cells (40 x)

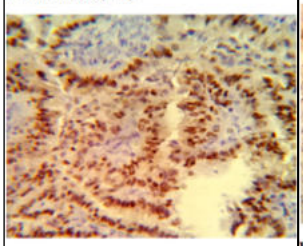


Figure 3- Section of Adenocarcinoma of prostate showing PR positivity in epithelial cells (40 x)

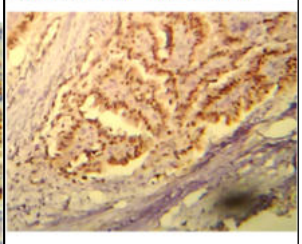


Figure 4 -Section of Adenocarcinoma of prostate showing PR positivity in epithelial cells and stromal cells (40 x)

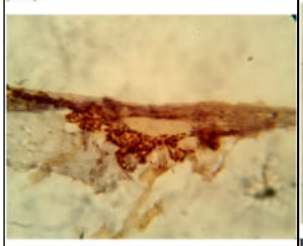


Figure 5- TRUS Biopsy of prostate showing PR positivity in epithelial cells (40 x)

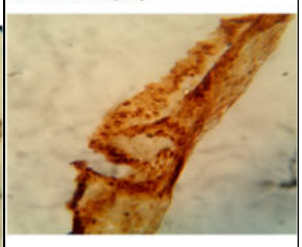


Figure 1- TRUS Biopsy of prostate showing PR positivity in both epithelial cells and stromal cells (40 x)

DISCUSSION

Different types of prostatic lesions are prevalent in general population mainly in elderly men such as BHP, Chronic prostatitis, PIN and prostatic carcinomas and presenting with urinary symptoms like

dysurea, incontinence, dribbling of urine, bladder outlet obstructions etc.[11] BHP is the most common lesion of prostate and it is neither a premalignant lesion nor a risk factor for malignancy but symptom complex of BHP usually have impaired quality of life especially in elderly men. So, both medical and surgical management is needed. [12]

In different type of prostatic lesions the serum level of Prostate Specific Antigen (PSA) is elevated. PSA is a 33kDa protein first identified in seminal fluid in 1971.[13] In 1979 Wang isolated PSA from prostatic tissue and described the chemical nature of it. It is a glycoprotein having 237 amino acid residues, 4 carbohydrate side chains and multiple disulfide bonds.[14] It is a serine protease and produced by prostate secretary epithelium and vesiculaeseminales.[15] Depending upon the molecular forms PSA is classified into three types. One is free PSA having molecular mass 30kDa, second is 780kDa protein bound to alpha-2-macroglobulin and last one is bound to alpha-anti-chymotrypsin having molecular mass 90kDa.[16]

Serum PSA level is dependent on age of the patient and prostatic volume, while volume of prostate is related to age. On the ground of that knowledge, screening of prostatic lesion in all age group males with the help of a single reference range is not ideal. Rather than a single cut off value, age-specific reference ranges of serum PSA are more discriminating tumour marker. It increases the specificity by detecting clinically significant prostatic carcinomas in old males and improves sensitivity, finding more potentially curable cancers in younger males.[17-19] **(Table1)**

Table 1 Age-specific Reference Range For PSA Values

Age range (years)	Oesterling et al[17]	Dalkin et al[18]	De Antonl et al[19]
40-49 years	0.0-2.5 ng/ml	-	0.0-2.4 ng/ml
50-59 years	0.0-3.5 ng/ml	0.0-3.5 ng/ml	0.0-3.8 ng/ml
60-69 years	0.0-4.5 ng/ml	0.0-5.4 ng/ml	0.0-5.6 ng/ml
70-79 years	0.0-6.5 ng/ml	0.0-6.3 ng/ml	0.0-6.9 ng/ml

In 1986 a US Food and Drug Administration (FDA) approved study conducted in Hybritech Inc., San Diego, CA including 6630 male aged 50-74 years, to established normal range of serum PSA <4.0 ng/ml and for early detection of prostatic lesions. According to this study prostatic biopsy was recommended for PSA > 4.0 ng/ml and lesions must be considered aggressive when PSA values >10 ng/ml.[20,21] Later different studies suggested that recommended value of serum PSA > 4ng/ml for biopsy might miss 20%- 40% of carcinoma.[22,23] To curtail this problem and increase carcinoma detection rate it is necessary to lower the cut-off value of serum PSA to earlier medical intervention to improve patient outcomes.[24,25] The 2.5 ng/ml cut-off was recommended by the National Comprehensive Cancer Network in the United States.[26] Lowering the cut-off value increases risk of identifying and unnecessarily treating indolent disease. Serum PSA value is used as a monitor of disease progression or recurrence. At the time of biopsy baseline serum PSA value is measured and over time subsequent change in value indicates disease progression, responsiveness of therapy or failure. American Joint committee on Cancer (AJCC) and Union Internationale Contre le Cancer (UICC) stratified the patients in four categories according the PSA concentration levels, < 4ng/ml, 4-10ng/ml, 10-20ng/ml and >20 ng/ml.[27]

Another two parameters PSA velocity and PSA density (PSAD) is used for screening of prostatic carcinomas with high accuracy. The calculation of PSAV was restricted to PSA values separated by at least 6 months and a maximum of 24 months.[28] The risk is calculated by counting the number of times that PSAV exceeded the cutoff point 0.4 ng/ml/year. Carter *et al* was counted original risk by using PSAV and this value was based from that study.[29] In another study Loeb Stacy *et al* demonstrated the association between a single value of PSAV >0.4 ng/ml/year with detection of carcinoma prostate.[30] In 2011 National Comprehensive Cancer Network was recommended a guideline to prostate biopsy. According to that prostatic biopsy was recommended for those who had total PSA levels ≤2.5 ng/ml and a PSAV ≥0.35 ng/ml/year.[31]

For the calculation of PSA density (PSAD) prostate volume is determined by transrectal ultrasound (TRUS) and PSA value (in ng/ml) is divided prostate volume (in cm^3). In a study Benson *et al* suggested the use of PSAD to discriminate prostate cancer from other causes of PSA elevation.[32] The recommended cut-off value of PSAD was 0.15 $mg/ml/cm^3$ which spared around 50% patients from undergoing unnecessary biopsies. However, few studies have reported that this cut off has low sensitivity.[33]

In this study mean value of serum PSA in patients presented with BHP was 3.62±1.22 ng/ml. A total 54 (88.52%) patients presented with serum PSA level below 4ng/ml. In BHP with chronic prostatitis, 13(65%) patients had serum PSA level in between 4ng/ml to 10ng/ml. A total of 6 patients were diagnosed with adenocarcinoma with mean serum PSA level was 102.2±58.62 ng/ml. Of them 5 cases (83.33%) presented with serum PSA level more than 10ng/ml and only one patient had PSA less then 10ng/ml. So our result showed that serum PSA values were much higher in carcinoma than that of BHP with *P* value 0.0001 and this result was also corroborated by the results of study by Aboseif *et al.*[34]

The homeostasis of prostate is maintained by reciprocal interactions in between epithelial and stromal cells. Aberrant stromal cell proliferation can disrupt this balance and result in diseases such as benign prostatic hyperplasia. Two isoforms of progesterone receptors (PR), PRα and PRβ are identified in prostate and these are expressed in stromal fibroblasts and smooth muscle cells but not in epithelial cells. They inhibit the expression of Cyclin A, Cyclin B, and CDC25C which leads to delaying the S and M phases of cell cycle. Both PRα and PR_{β} also regulate the activity different transcriptomes. All these properties of PR result in the suppression of prostate stromal cell proliferation.[35]

As compared to normal prostate the PR expression in stromal cells of malignant lesion decreases and it was demonstrated by immunohistochemistry by Y Yu *et al.* Their study suggested that invasion and migration of malignant cells of the prostate was inhibited by PR positive stromal cells. The secretion of stromal derived factor-1 (SDF-1) and interlukin-6 (IL-6) by stromal cells is suppressed by PR. The Stromal Derived Factor 1(SDF-1/CXCR4) stimulates the proliferation of malignant cells, promotes the invasion and angiogenesis and protect neoplastic cells from chemotherapeutic drug-induced apoptosis. IL6 also induces the proliferation by augmenting the activity of Janus Kinase/Signal Transducer and Activator Transcription 3 pathway in malignant cells. So decreased expression of the PR in stroma of malignant lesion may contribute to the elevated SDF-1 and IL-6 levels in prostate tumors and these factors enhance prostate tumor progression.[36] Stromal PR has inhibitory effect on benign prostatic hyperplasia (BHP), reactive stroma development, and prostatic carcinoma progression.[9]

In a study Helmut bankhoff *et al* suggested that the expression of ERα is restricted to the proliferation compartment (basal cells) of prostate while high levels expression of ERβ in the prostatic epithelium (secretory luminal cells). Cancerogenic effects of estrogens may be mediated by ERα on the dysplastic epithelium but ERβ is downregulated in HGPIN indicating the chemopreventive effects of estrogens. [37] In another study the majority cases of the epithelial and stromal component of prostatic carcinomas and BHP exhibited nuclear immunoreactivity for ERβ while they were negative for ERα.[38]

In our study immunohistochemistry was performed for demonstration of ER and PR in different type of prostatic lesions. In BHP cases, PR positivity was found in 51(83.6%) cases of epithelial cells and 53(86.88%) cases of stromal cells. A total 3(60%) cases of PIN cases showed epithelial and stromal cells positivity with PR. In adenocarcinoma patients PR positivity for epithelial and stromal cells were 2(33.33%) and 3 (50%) respectively. PR expression in epithelial cells in BHP cases was found to be is significantly higher than PR expression in tumour epithelial cells of adenocarcinoma with p value of 0.0149. In contrast to epithelial cells the expression of PR in stromal cells of BHP doesn't show any statistical significance to PR expression of stromal cell of adenocarcinoma with p value of 0.0512. The results of our study is very much in alignment with the study of M S Kang *et al,*[39] Hiramatsu *et al*[40] and Bankhoff *et al.*[41]

In our study ER in epithelial cells and stromal cells were negative in all cases. This result of our study is very similar to the study of M S Kang *et al,*[39] and M Wernet *et al.* [42]

Our study has few limitations too. Firstly the smaller sample size of study but it is due to limited study period. Secondly lack of serial follow up of serum PSA levels at proper interval of time by which we can calculate PSA velocity. We used only PSA in our study but PSAV is more sensitive to detection of prostatic lesions.[29] Lastly, IHC is a costly procedure so it can be considered as logistic limitation for this kind of study.

CONCLUSION

In our study, the expression of PR was present in most cases of BHP and almost half of carcinoma cases and expression of ER was negative in all cases. The serum level of PSA was significantly higher in carcinoma cases. These findings indicated that progesterone is strongly associated with different pathologies of prostate and antiprogesterone therapy may be helpful in the treatment of prostatic adenocarcinoma.

REFERENCES

1. Christine H. Lee, Oluyemi Akin-Olugbade, Alexander Kirschenbaum, Overview of Prostate Anatomy, Histology, and Pathology, Endocrinology and Metabolism Clinics of North America, Volume 40, Issue 3, 2011, Pages 565-575,
2. McNeal JE. Normal histology of the prostate. Am J Surg Pathol 1988;12(8):619–33.
3. Pavelić J, Zeljko Z. Prostate gland-transition zone lesions. Etiology, growth regulation, growth factors, genetic changes. Lijec□nic□ki Vjesn 2016;124(6-7):211–9.
4. Patel ND, Parsons JK. Epidemiology and etiology of benign prostatic hyperplasia and bladder outlet obstruction. Indian J Urol. 2014 Apr;30(2):170-6.
5. Guess HA, Arrighi HM, Metter EJ, Fozard JL. Cumulative prevalence of prostatism matches the autopsy prevalence of benign prostatic hyperplasia 1990;17(3):241–6.
6. Rekhi B, Jaswal TS, Arora B. Premalignant lesions of prostate and their association with nodular hyperplasia and carcinoma prostate. Indian J Cancer 2004;41(2):60–5.
7. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J cancer 2010;127(12):2893–917.
8. Bonkhoff H, Fixemer T, Hunsicker I, Remberger K. Estrogen receptor expression in prostate cancer and premalignant prostatic lesions. Am J Pathol 1999;155(2):641–7.
9. Chen RQ, Yu Y, Dong X. Progesterone receptor in the prostate: A potential suppressor for benign prostatic hyperplasia and prostate cancer. Journal of Steroid Biochemistry and Molecular Biology 2016;166:91-96
10. Prostate-specific antigen (PSA) best practice policy [Internet]. Cancer Network. 2000. Available from: https://www.cancernetwork.com/view/prostate-specific-antigen-psa-best-practice-policy.
11. Miah S, Catto J. BPH and prostate cancer risk. Indian J Urol 2014;30(2):214–8.
12. Men S, Cakar B, Conkbayir I, Hekimoglu B. Detection of prostatic carcinoma: the role of TRUS,TRUS guided biopsy, digital rectal examination, PSA and PSA density. J Exp Clin Cancer Res 2001;20(4):473–80.
13. Hara M, Koyanagi Y, Inoue T, Fukuyama T. [Some physico-chemical characteristics of " -seminoprotein", an antigenic component specific for human seminal plasma. Forensic immunological study of body fluids and secretion. VII]. Nihon hōigaku zasshi. Japanese J Leg Med [Internet]. 1971 Jul [cited 2016 Sep 15];25(4):322–4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/5106556
14. Wang MC, Valenzuela LA, Murphy GP, Chu TM. Purification of a human prostate specific antigen. Invest Urol 1979;17(2):159–63.
15. Sokoll LJ, Chan DW. Prostate-specific antigen. Its discovery and biochemical characteristics. Urol Clin North Am 1997;24(2):253–9.
16. Christensson A, Laurell CB, Lilja H. Enzymatic activity of prostate-specific antigen and its reactions with extracellular serine proteinase inhibitors. Eur J Biochem 1990;194(3):755–63.
17. Oesterling JE, Jacobsen SJ, Chute CG, Guess HA, Girman CJ, Panser LA, et al. Serum prostate-specific antigen in a community-based population of healthy men. Establishment of age-specific reference ranges. JAMA 1993;270(7):860–4.
18. Dalkin BL, Ahmann FR, Kopp JB, Catalona WJ, Ratliff TL, Hudson MA, et al. Derivation and application of upper limits for prostate specific antigen in men aged 50-74 years with no clinical evidence of prostatic carcinoma. Br J Urol. 1995 Sep;76(3):346–50.
19. DeAntoni EP, Crawford ED, Oesterling JE, Ross CA, Berger ER, McLeod DG, et al. Age- and race-specific reference ranges for prostate-specific antigen from a large community-based study. Urology. 1996 Aug;48(2):234–9.
20. Catalona WJ, Hudson MA, Scardino PT, Richie JP, Ahmann FR, Flanigan RC, et al. Selection of optimal prostate specific antigen cutoffs for early detection of prostate cancer: receiver operating characteristic curves. J Urol 1994;152(6 Pt 1):2037–42.
21. Catalona WJ, Smith DS, Ratliff TL, Dodds KM, Coplen DE, Yuan JJ, et al. Measurement of prostate-specific antigen in serum as a screening test for prostate cancer. N Engl J Med 1991;324(17):1156–61.
22. Mistry K, Cable G. Meta-analysis of prostate-specific antigen and digital rectal examination as screening tests for prostate carcinoma. J Am Board Fam Pract 2003;16(2):95–101.
23. Schröder FH, van der Cruijsen-Koeter I, de Koning HJ, Vis AN, Hoedemaeker RF, Kranse R. Prostate cancer detection at low prostate specific antigen. J Urol 2000;163(3):806–12.
24. Catalona WJ, Ramos CG, Carvalhal GF, Yan Y. Lowering PSA cutoffs to enhance detection of curable prostate cancer. Urology 2000;55(6):791–5.
25. Berger AP, Volgger H, Rogatsch H, Strohmeyer D, Steiner H, Klocker H, et al. Screening with low PSA cutoff values results in low rates of positive surgical margins in radical prostatectomy specimens. Prostate 2002;53(3):241–5.
26. De Angelis G, Rittenhouse HG, Mikolajczyk SD, Blair Shamel L, Semjonow A. Twenty Years of PSA: From Prostate Antigen to Tumor Marker. Rev Urol 2007;9(3):113–23.
27. Agnihotri S, Mittal RD, Kapoor R, Mandhani A. Raising cut-off value of prostate specific antigen (PSA) for biopsy in symptomatic men in India to reduce unnecessary biopsy. *Indian* J Med Res 2014;139(6):851-6.
28. Loeb S, Metter EJ, Kan D, Roehl KA, Catalona WJ. Prostate-specific antigen velocity (PSAV) risk count improves the specificity of screening for clinically significant prostate cancer. BJU Int 2012;109(4):508–13.
29. Carter HB, Kettermann A, Ferrucci L, Landis P, Metter EJ. Prostate-specific antigen velocity risk count assessment: a new concept for detection of life-threatening prostate cancer during window of curability. Urology 2007;70(4):685–90.
30. Loeb S, Roehl KA, Nadler RB, Yu X, Catalona WJ. Prostate specific antigen velocity in men with total prostate specific antigen less than 4 ng/ml. J Urol 2007;178(6):2348–53.
31. National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology. [Internet].https://www.nccn.org/professionals/physician_gls/pdf/prostate_detection.pdf
32. Benson MC, Whang IS, Pantuck A, Ring K, Kaplan SA, Olsson CA, et al. Prostate specific antigen density: a means of distinguishing benign prostatic hypertrophy and prostate cancer. J Urol 1992;147(3 Pt 2):815–6.
33. Lujan M, Paez A, Llanes L, Miravalles E, Berenguer A. Is there a role for this parameter when screening for prostate cancer? *Prostate Cancer* Prostatic Dis 2001;4(3):146-149.
34. Aboseif S, Shinohara K, Weidner N, Narayan P, Carroll PR. The significance of prostatic intra-epithelial neoplasia. Br J Urol 1995;76(3):355–9.
35. Yu Y, Liu L, Xie N, Xue H, Fazli L, Buttyan R, et al. Expression and function of the progesterone receptor in human prostate stroma provide novel insights to cell proliferation control. J Clin Endocrinol Metab 2013;98(7):2887–96.
36. Yu Y, Lee JS, Xie N, Li E, Hurtado-Coll A, Fazli L, et al. Prostate stromal cells express the progesterone receptor to control cancer cell mobility. PLoS One 2014;9(3):e92714.
37. Bonkhoff H, Motherby H, Fixemer T. New insights into the role of estogens and their receptors in prostate cancer. Urologe A. 2003 Dec.;42(12):1594–601.
38. Al-Maghrabi,J.A., Hassan, T.M., Abdel-Meguid, T.A. et at. Expression of estrogen alpha and beta receptors in prostate cancer and hyperplasia:Immunohistochemical analysis. Afr J Urol 16, 79-87(2010)
39. MS K, SY P, HK. Y. Estrogen and Progesterone Receptor Expressions in Benign Prostatic Hypertrophy and Prostatic Adenocarcinoma. Korean J Pathol 1998;32(5):346–51.
40. Hiramatsu M, Maehara I, Orikasa S, Sasano H.Immunolocalization of oestrogen and progesterone receptors in prostatic hyperplasia and carcinoma.Histopathology 1996;28(2):163–8.
41. Bonkhoff H, Fixemer T, Hunsicker I, Remberger K. Progesterone receptor expression in human prostate cancer: correlation with tumor progression. Prostate 2001;48(4):285–91.
42. Wernert N, Gerdes J, Loy V, Seitz G, Scherr O, Dhom G. Investigations of the estrogen (ER-ICA-test) and the progesterone receptor in the prostate and prostatic carcinoma on immunohistochemical basis. Virchows Arch A Pathol Anat Histopathol 1988;412(4):387–91.