Section A-Research paper

UTILITY OF ESTROGEN RECEPTOR BETA AND KI67 EXPRESSION IN BENIGN AND MALIGNANT PROSTATIC LESIONS

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Abstract

Background- Prostate cancer is the second most common cause of male cancer deaths. Recently studies have revealed the role of estrogen signaling pathways in the carcinogenesis of prostate. Estrogen regulates the growth of prostate through two receptors, ER-α and β, of which ER-β is proposed to be antiproliferative. It is noted that there is wide variation in results of various studies regarding the level of expression of ER β in benign and malignant prostatic lesions. There is need of additional research to standardize distribution of this receptor in human prostatic tissue.

Aim: To evaluate the immunoexpression of ER-β and Ki67 in benign and malignant prostatic lesions.

Methods: A hospital-based retroprospective study was conducted. Paraffin blocks of Benign Hyperplasia of Prostate(BPH) and carcinoma prostate were retrieved from Histopathology Section in the Department of Pathology. IHC evaluation of ER-β and ki67 was performed in these cases and the expression of these markers was compared between the two groups.

Results: 40 cases of BPH and 40 cases of Adenocarcinoma of prostate were included in the study. Comparison of the Immunohistochemistry marker ER-β and ki67 was done between the two study groups. Ki67 showed high sensitivity of 100% and specificity of 75% and ER-β showed sensitivity and specificity of 95% and 45% respectively and was statistically significant.

Conclusion: There was reduced ER-β expression in adenocarcinoma prostate when compared to Benign Hyperplasia. In contrast , Ki67 expression showed higher expression in carcinoma prostate.

Keywords – Adenocarcinoma of prostate, BPH, IHC, ER-β, Ki67.

Introduction

Prostate cancer accounted about 1,276,106 new cases and 358,989 deaths which is around 3.8% of deaths in men with cancer during the year 2018. The number however varies world wide. Based on the current research it is noted that the highest incidence of prostate cancer is widely seen in African – American population and higher probability of developing disease early in life as compared to other racial and ethnic groups.[1]

Initially it was assumed that in India the number of prostate cancer was very low as compared to the western globe but with increasing population in urban areas due to migration which led to change in lifestyles, easy access to medical facility & increased awareness the number of cases increased drastically. This clearly indicated that we would soon reach the numbers of western countries. Based on the cancer registries reports it is expected that we might face surge in cancer incidence in upcoming years.[2]

Localised prostate cancer is asymptomatic and is usually discovered by the detection of a suspicious nodule on rectal examination or elevated Serum prostate specific antigen(PSA) level. Patients with advanced prostatic cancer may present with symptoms of urinary obstruction. Typically, a transrectal needle biopsy is required to confirm the diagnosis.[3] The gold standard for the diagnosis of prostatic carcinoma is light microscopic findings, although there are complicated cases which would benefit from immunohistochemical studies.[4]

Immunohistochemistry (IHC) is a widely used ancillary testing method in anatomic surgical pathology for cell classification and diagnosis and utilizes antibodies targeted against certain antigens in specific tissues and cells to facilitate determination of cell type and organ of origin.[5]

Recently studies have revealed the role of estrogen signaling pathways in the carcinogenesis of prostate. Several phytoestrogens in the diet bind to estrogen receptors and activate detoxification enzymes such as glutathione-S-transferase in prostatic epithelium highlighting the chemo-preventive role of estrogen,while few experimental studies in animals have shown that chronic treatment of rats with testosterone leads to high incidence of prostate cancer when combined with estrogens. So this opposing effects exerted by estrogens on prostatic epithelium are proposed to be mediated by two types of receptors: ER α and ER β.[5]

ER α expression is limited to prostatic stromal cells and the basal cell layer, while secretory luminal cell types of the prostatic epithelium lack ER α at the mRNA and protein level.[7] Considering expression of ER β, its localization in human prostate tissue is not well recognized. There are conflicting results by different authors on its location in prostatic tissue. According to some authors ER β is highly expressed in rat prostate epithelial cells and in the secretory epithelium of normal human prostate, where the levels of ER β mRNA are higher than the levels of ER α mRNA[8]. A significant study by Leav et al. immunolocalized ER β predominantly in basal cells using a polyclonal antibody.[6]

As this receptor is proposed to be antiproliferative, a better understanding of the function of ER β in the evolution of prostate carcinoma could strongly impact on the therapeutic options for patients who have ER β expressing tumors. We conducted this study to find out the pattern of expression of ER β in prostatic carcinoma and prostatic nodular hyperplasia by immunohistochemistry and compare the results between the two groups. We also determined the proliferation index by Ki 67 immunoexpression in benign and malignant prostate lesions. As ER β is proposed to be antiproliferative and Ki 67 a marker of proliferation, we tried to find out the correlation between ER β and Ki 67 expression and ER β with the grade of the tumor, if any.[6]

Materials and methods

A prospective observational study was done on BPH and Adenocarcinoma of prostate (TURP specimen)in the Department of Pathology for a duration of 2 years.

Method of collection of data: Paraffin blocks of cases reported as Benign Hyperplasia of Prostate or Adenocarcinoma Prostate were retrieved from Histopathology Section in the Department of Pathology. All the clinical details, PSA levels and findings on imaging if any, were recorded. 40 cases each of Adenocarcinoma prostate and nodular hyperplasia prostate confirmed on histopathological examination were included in the study. Cases where tissue obtained was scant for immunostaining were excluded from the study.

Tissue for study included Transurethral Resection of Prostate chips. The tissue were preserved in 10% buffered formalin and processed routinely. Three 4µ-thick sections were prepared from each tissue paraffin block. One section was stained with Haematoxylin and Eosin (H & E) for morphologic diagnosis and Gleason's score and grade. Rest two sections were mounted on poly L lysine coated slides, which were subjected to ER β and Ki 67 immunohistochemical staining and interpretation of these markers was done.

IMMUNOHISTOCHEMISTRY EVALUATION OF ER β EXPRESSION:

Nuclear staining within the cell, whether strong or weak, were considered positive. A section of breast cancer was stained as a positive control. Immunostained slides were scored as for the routine evaluation of the ER status in breast cancer. First, a proportion score (PS) was assigned, which represents the estimated proportion of positive cells present in the entire slide. The PS included six categories ranging from 0 to 5, as follows: 0: none; 1: 1%; 2: >1–10%; 3: >10–33%; 4: >33–66%; 5: >66%. [9]

Next, an intensity score (IS) was assigned which represents the average intensity of positive cells and is scored as : 0: none; 1: weak; 2: intermediate; 3: strong. The PS and IS were then added to obtain a total score (TS) range from 0–8. For statistical analysis, the TS was subdivided into four categories including negative (TS, 0–2), weak (TS, 3–4), moderate (TS, 5–6), and strong (TS, 7–8).[9]

IMMUNOHISTOCHEMICAL EVALUATION OF KI-67 EXPRESSION

Antibody used for Ki 67: rabbit monoclonal prediluted antibody. A section of lymph node was stained as a positive control. The percentage of immunostained nuclei across the cancer areas were calculated and graded as follows: 1+ (<1 % stained nuclei) ,2+ (1–5 % stained nuclei), 3+(≥5–10 % stained nuclei), 4 (+≥10–20 % stained nuclei), 5+ (≥20 % stained nuclei). [6]

Results:

The age of patients with carcinoma prostate varied from 55-95 years with a median age of 70 years. Total number of cases included in the present study were 40 cases each of Benign Hyperplasia of Prostate and Adenocarcinoma of prostate. All prostatic adenocarcinomas (100%) were high grade tumors with a Gleason score of 7-10. Majority of the cases of BPH showed ER β immunoexpressionin the basal cell nuclei with only few cases showing nuclear positivity in stromal cells.(Fig 1)

In the present study,positive expression of ER β was noted in 77.5% of Benign hyperplasia cases in contrast to 30% cases of prostatic adenocarcinoma . Among the 77.5% of Benign hyperplasia cases, majority(37.5%) of them showed a total score of 4+ , while among 30% malignant cases withER βimmunoexpression, the majority (17.5%) of cases showed a total score of 2+ (Table 1) indicating a reduced expression in carcinomas.

A smaller number (2/40) of patients with adenocarcinoma prostate expressed ER β(Beyond

score-4+) at higher levels as compared to a large number (19/40) in the benign group.ER β expression was compared in two groups by Chi Square test and the result was statistically significant(*p* value <0.001).

In most of the benign cases (75%) in the present study, Ki67 expression was low(<5% , score 2) compared to adenocarcinoma of prostate. On the contrary, Proliferation index was higher in adenocarcinoma of prostate with a large number of cases (77.5%) showing values >10% (Score 4+,5+) (Fig. 2).Chi Square test was used to analyse the above data and *p* value was significant (<0.001)(Table 2)..

Discussion

Prostate cancer is primarily a disease of the elderly with more than three quarter of the cases occurring in men above 65 years of age. This disease has become a major health problem globally during the last few decades. It has been demonstrated and supported by studies that ER β is reduced in carcinoma Prostate compared to nodular hyperplasia as it is antiproliferative.[6]If the hypothesis that ER β is lost during carcinogenesis is true, it will help in guiding therapy in prostate cancer prevention trials. The results of different studies on localization and expression of ER βin prostatic lesions is quite variable.

Our results are in concordance with the study done by Leav et al.[6] who immunolocalized the receptor predominantly in basal cells of normal prostate and to some extent in stromal nuclei and secretory cells.In contrast to this finding, study done by Fixemer et al.[9] and Horvath et al.[10] suggested that this receptor is predominantly seen in secretory luminal cell types and to a lesser extent in basal cells.

The variable results on the localization of ER β in humans may be due to the different specificity of the primary antibodies used in different studies.[6]

We evaluated the levels of immunoexpression of ER-β in Benign hyperplasia of prostate and Adenocarcinoma prostate and compared them. 12 cases of carcinoma prostate in the present study showed a positiveER β expression though at much lower levels.Our results corroborate with study by Gabal SM et al.[11] who demonstrated diminished ER-Beta levels in carcinoma prostate as compared to Benign Hyperplasia.In contrast to our study, Horvath et al.[10] reported progressive loss of ER βin prostatic hyperplasia.

Contrary to the above findings Fixemer et al.[9] concluded that ER β levels are retained in all primary adenocarcinomas and metastatic carcinomas at high levels but reduced significantly in recurrent carcinoma. Eighty-seven percent of primary tumors in their study retained the high-level expression of the ER β whereas only 13 % revealed lower rates. There is no obvious explanation for these controversies between different reported results on the levels of ER β expression. It is well known that imperfect antibody specificity or different primary antibodies, ineffective antigen retrieval and tissue-processing methods, or the presence of unknown isoforms of ER protein may affect immunohistochemistry performance.

Role of ER β in prostate carcinogenesis has been emphasized through cancer prevention trials also. In one of the Prostate Cancer Prevention Trials, more than 18,000 healthy volunteers were randomly assigned to receive either finasteride or placebo. The incidence of tumors with a high Gleason grade was more in the finasteride group than in the placebo group. Finasteride is a drug that acts by suppressing ER β and preventing the differentiation of epithelium. This mechanism could account for the higher incidence of poorly differentiated tumors in the

finasteride group.[12]

Considering Ki 67, expression was higher in carcinoma prostate compared to benign hyperplasia. All of the adenocarcinoma cases (100%) had a Ki67 score of 3+ and above, ie a proliferation index >5% while most cases of BPH (75%) had score of 2+,ie a proliferation index between 1-5%. Our results are in concordance with other studies like Grover SK et al[5]showing increasedimmunoexpression of ki67 in adenocarcinoma prostate.

*Conclusion:*In this study,adenocarcinoma prostate showed reducedimmunoexpression of ER-β and higher Ki67 expression compared to Benign hyperplasia of prostate which showed reduced Ki67expression and higher ER-β expression.

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Tables/Figures

TABLE 1: ER BETA TOTAL SCORE BETWEEN STUDY GROUPS

ER BETA TOTAL SCORE	MALIGNANT CASES		BENIGN CASES		p value
	N	%	N	%	
0	28	70.0	9	22.5	<0.001*
2	7	17.5	4	10.0	
3	3	7.5	9	22.5	
4	1	2.5	15	37.5	
5	1	2.5	0	0.0	
6	0	0.0	1	2.5	
8	0	0.0	2	5.0	
Total	40	100.0	40	100.0	

Note: p value* significant at 5% level of significance (p<0.05)

TABLE 2: DISTRIBUTION OF KI-67 (PERCENTAGE) AND KI67 SCORE BETWEEN STUDY GROUPS

KI-67 % (Score)	MALIGNANT CASES		BENIGN CASES		p value
	N	%	N	%	
<5 (2)	0	0	30	75	0.001*
>5-10 (3)	9	22.5	10	25	
>10-20 (4)	16	40	0	0	

>20 (5)	15	37.5	0	0	
Total	40	100	40	100	

Note: p value* significant at 5% level of significance ($p<0.05$)

Figure Legends:

Fig 1: IHC Staining showing positive ER-β immunoexpression in myoepithelial cells and few stromal cells (400x)

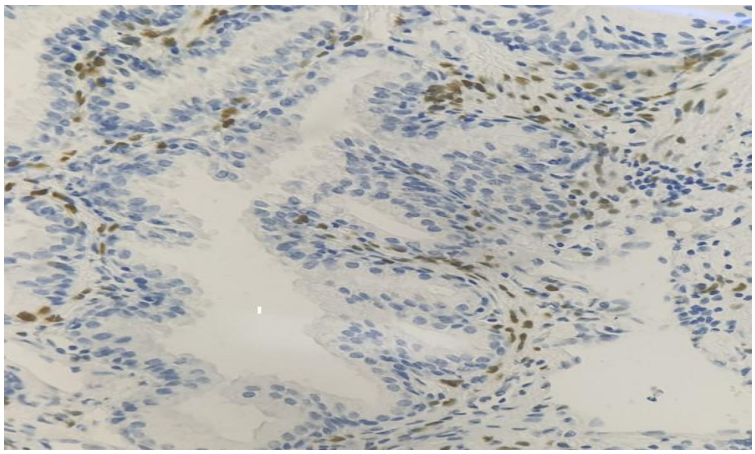

Fig 2: IHC staining showing ki67 positivity with >20% proliferative index(400x)