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Original Research Article

A STUDY ON EVALUATION OF IMMUNOEXPRESSION OF ER β AND KI67 IN BENIGN AND MALIGNANT NEOPLASMS OF PROSTATE

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ABSTRACT

Background: Prostate cancer is one of the commonest cancers and cancer related morbidity and mortality in males. Early identification of type of biomarkers like ER β and Ki67 can identify the aggressiveness of tumor and guide in targeted chemotherapy.

Materials and Methods: this observational study was done in the Department of Pathology, Malla Reddy Medical College for Women, over a period of 1 year, i.e. from August 2023 to July 2024. A total of 50 patients, consisting of 25 patients with BPH and 25 patients with prostate cancer were included in this study.

Results: levels of ER β expression were found to be higher in patients with BPH and a small proportion of patients with prostatic cancer. ER β was predominantly expressed from the secretory epithelium. High levels of Ki67 were predominantly expressed in patients with prostate cancer and reduced levels of ki67 were expressed in patients with BPH.

Conclusion: Understanding the intricacies of estrogen signaling in prostate cancer may provide insights into novel therapeutic strategies and improve patient outcomes.

Keywords: prostate, benign prostatic hypertrophy, prostate cancer, ER β, ki67, immunotyping.

INTRODUCTION

Prostatic cancer is the second most common cancer in males worldwide. Previously, prostate cancer was considered an androgen-driven disease, primarily influenced by the androgen receptor (AR). However, recent evidence suggests that estrogen signaling, mediated through Estrogen receptors alpha (ERα) and beta (ERβ), also play a significant role in prostate cancer development and progression. ERα and ERβ are expressed in both normal and malignant prostate tissue, and their activation can lead to increased cell proliferation, potentially contributing to tumor growth.[1]

Estrogen signaling exerts anti-apoptotic effects in prostate cancer cells, promoting cell survival and resistance to cell death pathways.[2] Estrogen can modulate AR expression, AR-mediated transcription, or even act independently to drive prostate cancer progression.[3]

Estrogen receptors have been implicated in promoting angiogenesis, the formation of new blood vessels, within prostate tumors. Further, estrogen receptors mediated signaling may promote the metastatic potential of prostate cancer cells by accentuating epithelial to mesenchymal transition (EMT) and enhancing cell motility and invasion.[4]

Ki-67 is a novel proliferative marker that is expressed in all stages of the cell cycle, except G0, as the resting cells entering from G0 lack Ki-67 in the early part of G1.[5] This makes it an excellent marker for determining the proliferative potential of a tumor. Ki67 scoring is essential for diagnosis of tumor grade, based on proportion of tumor-positive cells.[6,7]

This study was conducted with an aim to evaluate the expression of ki67 and ERβ in patients with benign and malignant prostatic lesions.

MATERIALS AND METHODS

This prospective study was conducted in the Department of ¬¬Pathology, Malla Reddy Medical College for Women, over a period of 1 year, i.e. from August 2023 to July 2024. A total of 50 cases diagnosed with either benign hyperplasia prostate or carcinoma prostate were taken from Department of surgery.

A detailed history was taken from the patients regarding the complaints, clinical findings. Basic hematological tests were done for all 50 patients. PSA levels and necessary radiological investigations were done. 25 cases each of carcinoma prostate and nodular hyperplasia prostate/benign prostatic hypertrophy, confirmed on histopathological examination were included in the study.

The tissue was preserved in 10 % buffered formalin. Sections for histopathological examination were prepared from the tissue block and stained with Haematoxylin and Eosin (H & E) stain (for morphologic diagnosis and Gleason's score) and immunohistochemical staining for ERβ and Ki 67.

Evaluation of Estrogen Receptor β Expression: ERβ is a steroid receptor present within the nucleus. Hence, cells with nuclear staining were considered to be positive. The number of cells with nuclear staining were counted in each batch of 400 cells to determine the percentage of ERβ positive cells. Scores were expressed as the percentage of cells demonstrating nuclear immunoreactivity. 1+ = <10 %; 2+ = 11–40 %;3+= 41– 60 %; 4+ = 61–80 % and 5+ = >80 % nuclear positivity for ERβ.

Immunohistochemical Staining for Ki 6: Similar procedure was followed for evaluating proliferative index by Ki 67 immunostaining. The percentage of immunostained nuclei across the cancer areas was calculated and graded as follows: 1+ = when <1 % of cells are positive; 2+ = when 1-5 % of cells are positive; 3+ = when ≥5–10 % of cells are positive; 4+ = when ≥10–20 % of cells are positive and 5+ = when ≥20 % of cells are positive.

Statistical Analysis

IHC profile for ERβ and ki67 was compared between the patients with benign prostatic lesion (n=25) and malignant prostatic lesion (n=25) using Pearson chi square test and Fischer exact test. p value <0.001 was considered significant.

RESULTS

This study was conducted including 25 patients each with benign prostatic hypertrophy and prostatic carcinoma. Patients with benign prostatic hypertrophy were from age 50 years to 80 years with a median age of 69.6 years and patients with carcinoma prostate were from 47 to 82 years of age with a median of 71.2 years.

Patients with BPH had mild elevation of PSA levels (<10ng/ml, while patients with carcinoma prostate had elevated serum PSA levels ranging from 10 to 649 ng/ml. the mean PSA value of study population was 457.5 ng/ml.

Table 1: ERβ levels in BPH and carcinoma

score	Benign	malignant
1	1	9
2	1	5
3	2	4
4	10	4
5	11	3
total	**25**	**25**

On histopathological examination, all prostatic carcinomas were adenocarcinomas and majority (52%; n = 13) were high grade tumors with a Gleason score of 8–10.

84% of the patients with benign prostatic hypertrophy and 28% of patients with carcinoma prostate expressed ERβ at high levels (scores of 4+ and 5+). While, 72% of patients with prostate cancer had relatively low levels of ERβ expression (scores of 1+, 2+ and 3+). ERβ expression was compared in two groups and the difference was statistically significant (p value< 0.001).

Proliferative index measured by the levels of Ki67 expression was below 5% in most of the cases with benign prostatic hypertrophy (n=21; 84%). On the contrary, majority of the patients with prostatic cancer (n = 15; 53.3%) had higher levels of proliferative index (>10%). Ki67 expression was compared between two groups and the difference was statistically significant (p value was significant (<0.001).

Table 2: Ki67 levels in BPH and prostate cancer

Scores	Benign	Malignant
1+	5	3
2+	16	4
3+	1	3
4+	2	5
5+	1	10
Total	25	25

Correlation between expression of Ki 67 with ER β in the two study groups (benign and malignant prostate neoplasm) was measured using the Pearson correlation but the results were found to be insignificant (p value >0.05). No correlation was found between the Gleason score and levels of ER β expression.

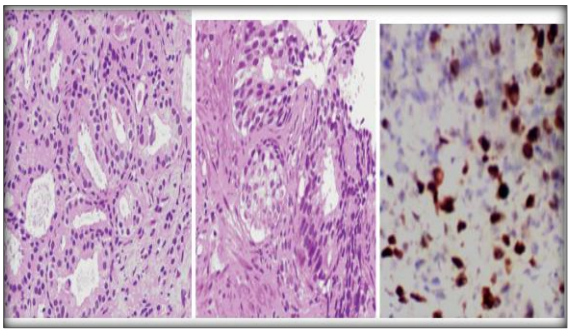

Figure 1-3: H & E staining of prostate carcinoma (fig 1,2). Ki67 staining of prostate cancer (fig 3)

DISCUSSION

This observational study was done in the Department of ¬¬Pathology, Malla Reddy Medical College for Women, over a period of 1 year including 50 patients. Amongst the 25 patients with prostate cancer, adenocarcinoma was the most common type observed in present study.

In present study, BPH was found in age between 50 years to 80 years and carcinoma prostate was found in age between 47 to 82 years of age. According to the American Cancer Society report (2016), prostate cancer is very rare below 40 years of age and upto 60% of all prostate cancer cases are diagnosed in men aged above 60 years.[8]

In present study, ERβ were expressed from stromal epithelial cells which is in concordance with study done by Fixemer and Horvath et al,[9,10] However, according to a major study by Leav et al,[11] ERβ expression is highest from the basal cells and upto some extent from the stromal nuclei. This variability could be explained due to the difference in the specificity of antibodies used. Leav et al, in their study used the GC-17 antibody which was prepared against the F domain of ER β, that identifies a post-transcriptionally modified form of the long-form ER β. Whereas in present study, the long and the short form of the ER β is directed against synthetic peptide derived from the C terminus of the human Estrogen receptor β 1.

In present study, the levels of immunoexpression of ER β in benign prostatic hyperplasia and carcinoma prostate was compared. Expression of ERβ was found to be lower in patients with prostate cancer than that of in patients with benign prostatic hypertrophy. According to few studies, due to its anti-proliferative actions, anti-invasive and pro-apoptotic properties, ERβ levels are lower in carcinoma of prostate than in BPH.[11-13] A possible implication of ER β in neoplastic growth control is supported by the findings of a selective loss of ER β protein in colon adenocarcinoma and ovarian cancer.[14,15]

Majority of patients with BPH had levels of ERβ expression while majority of patients with prostate cancer had low levels. Similar results have been given in another recent study by Asgari M et al,[16] and Gabal SM et al,[13] where majority of (92.1 %) cases of carcinoma prostate showed positive ER β expression. Similarly Horvath et al,[10] also reported progressive loss of ER β in prostatic hyperplasia and to a greater extent in invasive cancer. Only 11 % of carcinoma patients in their study expressed ER β at levels >5 %. However, contrary to the above findings, Fixemer et al.9 concluded that ER β levels are at high levels in all primary adenocarcinomas and metastatic carcinomas (similar to BPH) but reduced significantly in recurrent carcinoma.

According to few studies, the basal cell layer is responsible for cellular proliferation in normal prostate gland. As a result, this layer has lower levels of ER β expression. However, the secretory epithelium of prostate has higher levels of ER β, thereby indicating its anti-proliferative role.[17]

In present study and study done by Fixemer et al,[9] there was no correlation between Gleason score and expression of ER β. Leav et al. reported loss of ER β expression in high-grade dysplasia and its reappearance in grade three cancers.[10]

Ki67 is a proliferative tumor marker and high levels of its expression indicate the aggressiveness of the tumor. In present study, Ki67, expression was higher in prostate carcinoma than BPH. This is supported by studies done by Nornazirah et al,[18] and Renuka et al,[19] and Mohamed et al.20 Majority of cases with carcinoma had proliferation index >10 % while most cases of BPH had proliferation index between 1 and 5 %. Our results are in accordance with other studies on immunoexpression of Ki 67 in prostatic neoplasia.[21] However, there is no correlation between immunoexpression of Ki 67 and ER β in present study

CONCLUSION

Prostate cancer is one of the most common cancers in males worldwide. Immunoexpression of biomarkers like ER β and ki67 can identify the proliferative potential and aggressiveness of prostate tumor. This study concludes that there is higher expression of ER β in BPH and a small proportion of prostate cancer, while a majority of patients with prostate cancer have low levels of ER β expression. On the contrary, levels of Ki67 expression are higher in patients with prostate cancer than in patients with BPH.

Assessment of the levels of these biomarkers might have an implication during treatment of prostatic carcinoma as therapy targeting these receptors could be a part of treatment protocol.

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