Research Article

EXPRESSION OF BETA ESTROGEN RECEPTOR IN BENIGN PROSTATIC HYPERPLASIA AND ADENOCARCINOMA OF PROSTATE

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ARTICLE INFO	ABSTRACT
	In aging men, benign prostatic hyperplasia (BPH) and prostate adenocarcinoma (PCa) are the most common prostatic diseases. These diseases are androgen-dependent and are treated by inhibiting androgens or their action. However recent studies have shown that prostate growth is also influenced by estrogen. Estrogen induction of cell proliferation which plays a crucial role in hormone dependent tumors like breast and uterus is now also thought to play a significant role in normal and abnormal growth of the prostate gland. The role of estrogen receptor Beta (ER) in the pathogenesis or prognosis of PCa is unclear, it seems to have a role in the control of proliferation and the prevention of hyperplasia. This study was initiated to determine the expression of ER beta in BPH and PCa as only few studies had been done. Twenty nine cases each of TURP specimens of BPH and PCa were included in the study. The tumors were categorized as low, intermediate and high grade on their gleasons score. In BPH cases 93.1% of them were ER beta positive and 6.9% were ER beta negative. However in adenocarcinoma group only 3.4% were ER beta positive and 96.6% were ER beta negative. This observation was statistically significant. Elderly age group is most commonly affected with BPH and Adenocarcinoma of prostate. Based on the results of the present study, ER beta seemed to play a definitive role in the carcinogenesis. Estrogen beta is hypothesized to be involved in the carcinogenesis neoplastic progression of adenocarcinoma of prostate and underlying pathogenesis of BPH.

INTRODUCTION

In aging men, benign prostatic hyperplasia (BPH) and prostate adenocarcinoma (PCa) are the most common prostatic diseases (Garraway WM *et al*, 1991). BPH commonly arises from the transitional zone where stromal and epithelial nodules are most likely to develop, whereas PCa arises in the peripheral zone of the prostate gland where mostly epithelial cells undergo malignant transformation.

These diseases are androgen-dependent and are treated by inhibiting androgens or their action. However recent studies have shown that prostate growth is also influenced by estrogen (Al Maghrabi JA *et al*, 2010).

Estrogen induction of cell proliferation which plays a crucial role in hormone dependent tumors like breast and uterus is now also thought to play a significant role in normal and abnormal growth of the prostate gland (Kuiper GG *et al*, 1996).

The detection of two types of estrogen receptor and has brought new insight into the mechanism underlying estrogen signaling. Estrogen beta is found to be expressed in epithelium of the normal prostate gland. The role of estrogen receptor Beta in the pathogenesis or prognosis of PCa is unclear, it seems to have a role in the control of proliferation and the prevention of hyperplasia (Torlakovic E *et al*, 2002).

The expression of ER beta in BPH and PCa may provide an insight into the carcinogenesis of PCa. Though antiestrogen was earlier used in therapy of prostatic lesions, it was discontinued due to its side effects. The detection of a new ER beta and its expression may also help in providing newer therapeutic modalities for BPH and androgen resistant PCa with recurrence. Only few studies determining expression of ER beta has been done in prostate. Hence the study is initiated to determine the expression of ER beta in BPH and PCa.

Objectives of the Study: To determine the expression of estrogen receptor beta in BPH and adenocarcinoma of prostate and correlate with different grades of prostatic adenocarcinoma.

MATERIALS AND METHODS

29 cases each of TURP specimens of BPH and PCa received in the Department of Pathology R.L.Jalappa Hospital and

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Research Center attached to Sri DevarajUrs Medical College, Tamaka, and Kolar from November 2014 to October 2016 was included in the study. Cases with history of prior radiotherapy and chemotherapy were excluded from the study. Data regarding the clinical detail and serum PSA levels were collected. H and E stained slides were reviewed for the grading and scoring of Gleason's score. The tumors were categorized as low grade if Gleason score was equal to or less than 4 and high grade if Gleason score was equal to or more than 8. Gleason score of 5, 6 and 7 will be considered as intermediate. Immunostaining for ER beta was performed on all cases of BPH and PCa using appropriate positive and negative controls

Immunohistochemistry

It was done using primary antibody ER beta (Biogenex,) to know the activity in all the cases.

Sections are cut at approximately 3-4μm thickness, floated on 4% organosialine coated slides and incubated at 58°C over night. Deparaffinization using Xylene I and II for 15 minutes each. Dexylenisation using absolute alcohol I and II for 1 minute each. Dealcoholisation using 90% and 70% alcohol for 1 minute each. Tap water wash for 10 minutes followed by distilled water wash for 5 minutes. Antigen Retrieval technique was done with microwave at power 10 for 6 minutes in EDTA TRIS buffer pH 9.0 for 3 cycles. Distilled water rinsing for 5 minutes. Transfer to TBS (Tris buffer solution pH-7.6) for 5 minutes washing Peroxidase block: 10-15 min to block endogenous Peroxidase enzyme using 3% Hydrogen peroxide. TBS buffer wash thrice for 5 minutes. Power block: 10-15 mins to block non-specific reaction with the other tissue antigen.

Primary stain: Drain and cover the sections with ready to use Biogenex primary antibody ER beta antibody for 2 hours, followed by TBS buffer wash for 5 minutes twice to wash unbound antibodies. Super sensitive poly–horse radish peroxidase (HRP) for 30 minutes– to elongate chain and also to label the enzyme. Followed by TBS buffer wash for 5 minutes thrice to wash unbound antibodies. Color development with working DAB solution for 5-8 minutes, which imparts color to the antigens. TBS buffer wash for 5 minutes thrice then tap water wash for 5 minutes.

Counter stain Hematoxylin for 2 seconds followed by tap water wash for 5 minutes to wash out the excess stain. Dehydration and clearing by Alcohol: Xylene for 2 minutes. Then the slides were mounted with DPX.

Immunohistochemical evaluation

The immunostained sections were examined using light microscopy Estrogen receptor beta expression was thoroughly sought for in tumoral cells for nuclear staining.

Slide was examined under Low power (40 x) for area of maximum positivity.

Areas showing maximum positivity were chosen, and 200 cells were counted under High Power (400x).

Estrogen receptor-beta expression was assigned as positive when more than 10% of tumoral nuclei are stained, according to Agsari *et al* (2011). Rate of ER- expression was defined as percentage of positive nuclei per 200 cells counted. Intensity of staining was not taken into consideration, weak and strong staining was considered positive (Mann S *et al*, 2001)

ANALYSIS AND RESULTS

Statistical analysis

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test of Fischer's exact test (for 2x2 tables only) was used as test of significance for qualitative data. Yates correction was applied were ever chi-square rules were not fulfilled (for 2x2 tables only).

RESULTS

Mean age of subjects in BPH group was 54.1 ± 5.3 years in Adenocarcinoma subjects was 63.9 ± 6.5 years.In BPH subjects 89.7%of them had PSA 10ng/ml and 10.3% had PSA > 10ng/ml. were as in Adenocarcinoma subjects 100% of them had PSA > 10ng/ml. This observation was statistically significant. In Adenocarcinoma subjects, 37.9% had high, 58.6% had intermediate and 3.4% had low Gleason grade

Table 1 ER Beta findings comparison between two groups

		Group			
		BPH		Adenocarcinoma	
		Count	%	Count	%
ER Beta staining	Negative (10)	2	6.9%	28	96.6%
	Positive (>10)	27	93.1%	1	3.4%

$\chi^2 = 46.67$, df = 1, p <0.001*

In BPH subjects 93.1% of them were ER beta positive and 6.9% were ER beta negative. In Adenocarcinoma group only 3.4% were ER beta positive and 96.6% were ER beta negative. This observation was statistically significant. In BPH group among subjects with PSA 10ng/ml, 100% of them were positive for ER beta and among subjects with PSA >10ng/ml, 66.7% were negative and 33.3% were positive for ER beta. This observation was statistically significant. In Adenocarcinoma subjects with high and intermediate Gleason grade being 11 and 17 cases respectively, 100% were negative for ER beta, and 100% of subjects with low grading were positive for ER beta. This observation was statistically significant.

DISCUSSION

In the present study, ER beta positivity was seen in 93.1% of the cases, while loss of expression in only 6.9% of the BPH cases. These finding were similar to studies by Gabal *et al* (2007), which showed ER beta positivity in majority cases of BPH and Al –Maghrabhi *et al* (2010) which showed ER beta positivity in 94.3% of the BPH cases and Grover *et al*(2014), showed ER beta positivity in 100% of the BPH cases. Fixenar *et al* (2003) study concluded that ER beta levels were high in all BPH.

However few studies showed contradictory findings such as Hovarath *et al*(2001) which stated the progressive loss of ER beta in BPH with ER beta positivity seen only in 24.2% of BPH cases.

Table 2 The ER-*β* expression in the studied BPH cases

	Present Study	Horvath *et al* (2001)	Gabal *et al* (2007)	Al– Maghrabhi *et al* (2010)	Grover *et al* (2014)
ER BETA (+) (>10)	93.1%	24.2%	70%	94.3%	100%
ER BETA(-) (10)	6.9%	75.8%	30%	5.7%	0%

Table 3 The ER-*β* expression in the studied Adenocarcinoma cases

	ER-*β*(+) (>10)	ER-*β*(-) (10)
Present study	3.4%	96.6%
Gabal *et al* (2007)	17.2%	82.8%
Horvath *et al* (2001)	11.3%	88.7%
Grover *et al* (2014)	96.6%	3.4%
Al – maghrabhi *et al* (2010)	93.8%	6.2%
Asgari m *et al* (2011)	92.1%	7.9%
Fixemer *et al* (2003)	87%	13%
Torlakovic *et al* (2002)	93%	7%

Estrogens are believed to play a vital role in the pathogenesis of prostate adenocarcinoma cancer (PCa).

Although the precise biological function of ER-*β* is incompletely understood, it has been suggested that the receptor, acting through estrogens, may protect the normal prostate epithelium from undergoing cell proliferation, neoplastic transformation, and from oxidative injuries.

In present study, majority of the adenocarcinoma cases 96.6% were negative for ER beta expression. These finding were similar to the findings of Gabal *et al* (2007), which concluded that 82.8% of adenocarcinoma cases showed loss of ER beta expression while Horvath *et al* (2001) stated 88.7% of the adenocarcinoma of prostate cases showed loss of ER beta expression. Also in the present study, there is progressive loss of ER beta expression in different grades (Gleason) of adenocarcinoma of prostate, with low grades (2 -4) showing ER beta positivity in 100% cases and loss of ER beta expression in intermediate (5-7) and high grades (8-10) of adenocarcinoma of prostate. This ER beta is inversely proportional to grades of adenocarcinoma of prostate.

Leavakov *et al* (2015) study concluded that that 87% of low grade adenocarcinoma of prostate showed ER beta positivity while only 20% of the intermediate grade adenocarcinoma of prostate showed ER beta positivity. These finding were similar to the present study.

However many studies discussed below had findings different from that of our study. Though Grover *et al* (2014), study concluded that majority of the PCa cases were positive for ER expression, all his BPH cases were not only positive but also had higher level of ER expression concluding that in PCa ER though positive shows lower scores than BPH.

Asgari M *et al* (2011), study stated 92.1% cases of adenocarcinoma of prostate showed ER beta positivity which was different from the present study.[5]

AL-Maghrabhi *et al* (2010), study concluded that ER beta positivity was seen in 93.8% of adenocarcinoma of prostate and did not show any correlation between Gleason score and ER Beta expression which was different the present study.

Fixemer *et al* (2003) study concluded that ER beta levels were high in all Adenocarcinoma of Prostate, with majority showing high level expression that is 87% of the adenocarcinoma cases were as ER beta was reduced significantly only in recurrent carcinoma.
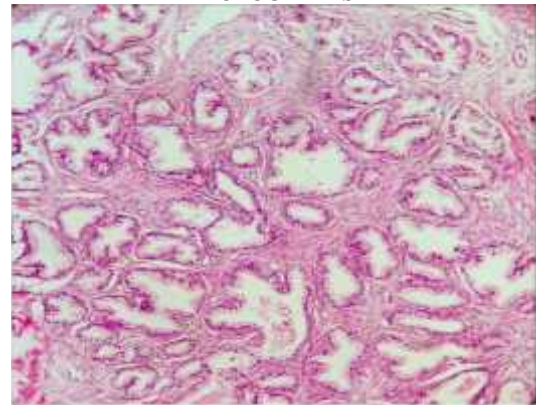
PHOTOGRAPHS
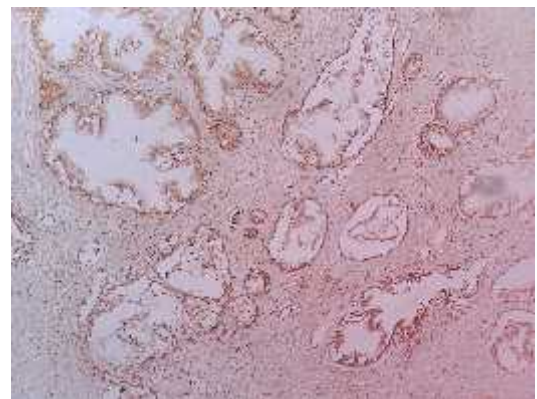

Figure-1 BPH (H&E, x400)

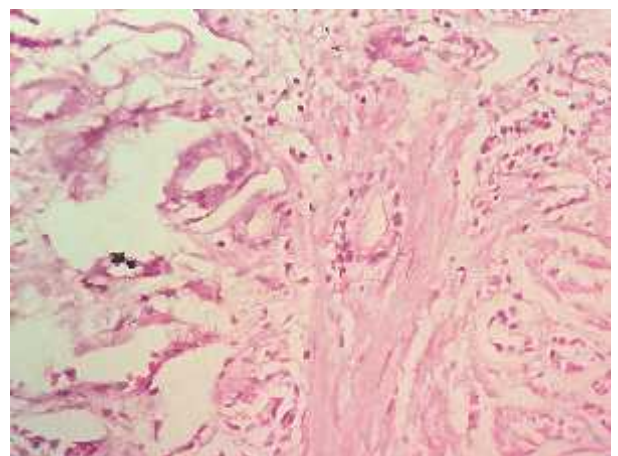**Figure-2** BPH, ER beta Positive (IHC ER beta, x400)

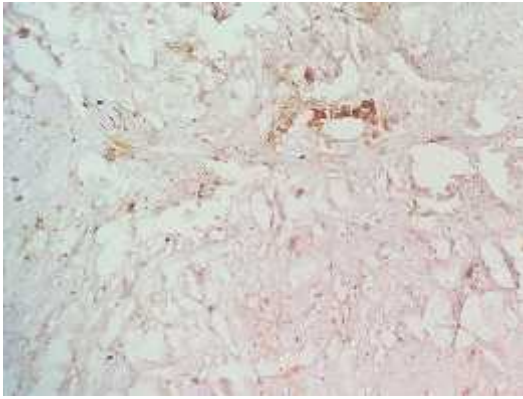**Figure-3** Prostatic Adenocarcinoma Gleason Score - 4 (H&E, x400)

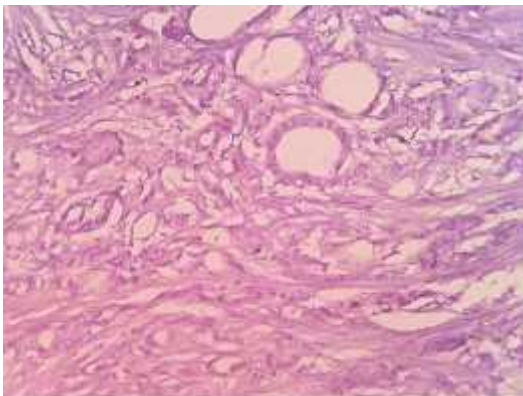**Figure-4** Prostatic Adenocarcinoma Gleason Score - 4, ER beta Positive
(IHC ER beta, x400)

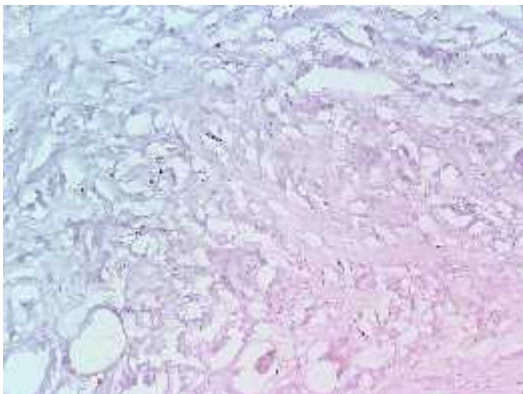**Figure-5** Prostatic Adenocarcinoma Gleason Score - 7 (H&E, x400)

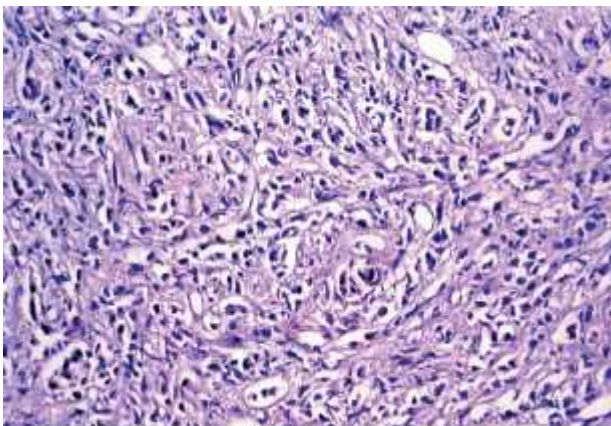**Figure-6** Prostatic Adenocarcinoma Gleason Score - 7, ER beta Negative
(IHC ER beta, x400)

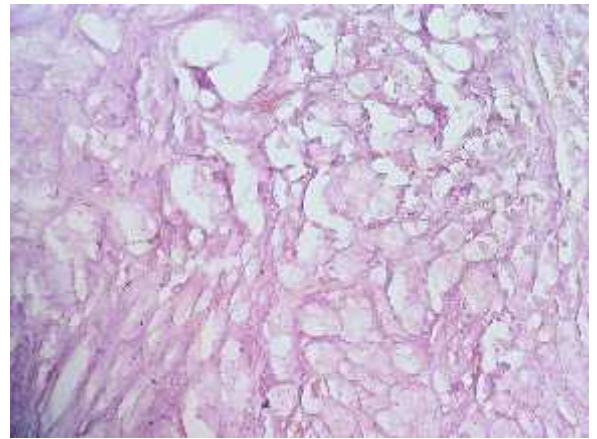**Figure- 7** Prostatic Adenocarcinoma Gleason Score - 10 (H&E, x400)

Figure-8 Prostatic Adenocarcinoma Gleason Score - 10, ER beta
Negative (IHC ER beta, x400)

Torlakovic *et al* (2002), stated that ER beta was expressed in 93% of adenocarcinoma of prostate and was associated positively with primary Gleason grade.[4]

Reason for contradicting results in the above studies can be due to the method used for ER detection, clone of antibody used, the different processing techniques and in some studies different cut off used for positivity and negativity of the marker.

As we had hypothesized our finding strongly suggest that ER beta has antiproliferative role in carcinogenesis of prostate where as ER alpha has a proliferative role on prostatic epithelium. Based on these finding, of the present study was ER beta will be present in non neoplastic and benign lesions compared to neoplastic lesion. Therefore explaining the protective role of ER beta in prostate.

CONCLUSION

Elderly age group is most commonly is affected with BPH and Adenocarcinoma of prostate. Our result shows majority of the BPH and all low grade PCa positive for ER beta and all the intermediate and high Gleason grade PCa negative for ER beta. Based on the results of the present study, ER beta seem to play a definitive role in the carcinogenesis. Estrogen beta, is hypothesized involved in the carcinogenesis and neoplastic progression of adenocarcinoma of prostate and underlying pathogenesis of BPH.

Futher scope of the study: However, multicentric and further genetic work up needs to be done, with larger sample size, to confirm the hypothesis so that it can be used for therapeutic management by antiestrogen beta treatment as a part of personalized treatment.

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