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

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INTRODUCTION

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 β) 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.

MATERIALS AND METHODS

29 cases each of TURP specimens of BPH and PCa received in the Department of Pathology R.L.Jalappa Hospital and
Research Center attached to Sri Devaraj Urs 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 3-4 m thickness, floated on 4% organosialine coated slides and incubated at 58°C overnight. Deparaffinization using Xylene I and II for 15 minutes each. Deyxilenisation using absolute alcohol 1 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-β 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. Adenocarcinoma cases 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 %             | Negative (≤10)                    | Positive (>10)       |
| ER Beta staining    | 2                                 | 28                   |
|                     | 6.9%                              | 96.6%                |
|                     | 93.1%                             | 3.4%                 |

χ² = 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–Maghrabi 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.
Estrogens are believed to play a vital role in the pathogenesis of prostate adenocarcinoma cancer (PCa). Although the precise biological function of ER-ß 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 ERß expression, all his BPH cases were not only positive but also had higher level of ERß expression concluding that in PCa ERß 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.

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.

Table 2 The ER-ß expression in the studied BPH cases

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

Table 3 The ER-ß expression in the studied Adenocarcinoma cases

| ERß(+) | Present study | Gabal et al (2007) | Horvath et al (2001) | Grover et al (2014) | Al– maghrabhi et al (2010) | Asgari m et al (2011) | Fixemer et al (2003) | Torlakovic et al (2002) |
|---------|---------------|--------------------|----------------------|---------------------|-----------------------------|-----------------------|-----------------------|------------------------|
| (≥10)   | 3.4%          | 96.6%              | 3.4%                 | 17.2%               | 82.8%                        | 11.3%                 | 96.6%                 | 93.8%                  |
| (≤10)   | 96.6%         | 3.4%               | 88.7%                | 88.7%               | 6.2%                         | 7.9%                  | 3.4%                  | 7%                     |

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)
Torlakovic et al. (2002), stated that ER beta was expressed in 93% of adenocarcinoma of prostate and was associated positively with primary Gleason grade.5

Reason for contradicting results in the above studies can be due to the method used for ER β 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.

BIBLIOGRAPHY

1. Garraway WM, Collins GN, Lee RJ. High prevalence of benign prostatic hypertrophy in the community. Lancet 1991; 338:469 -71.
2. Al Maghrabi JA, Hassan TM, Abdel Meguid TA, Mosli HA. Expression of Estrogen Alpha and Beta Receptors in Prostate Cancer and Hyperplasia: Immunohistochemical Analysis. Afr J Urol 2010; 16:79-87.
3. Kuiper GG, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA* 1996;93:5925-30.

4. Torlakovic E, Lilleby W, Tarlokovic G, Fossa SD, Chibbar R. Prostate carcinoma expression of estrogen receptor-a as detected by PPG5/10 antibody has positive association with primary Gleason grade and Gleason score. *Hum Pathol* 2002; 33:646-51.

5. Asgari M, Morakabati A. Estrogen Receptor beta expression in prostate adenocarcinoma. *Diagn Pathol* 2011;6:61.

6. Mann S, Laucirica R, Carlson N, Carlson N, Younes PS, Ali N, Younes A, Li Y, Younes M. Estrogen receptor beta expression in invasive breast cancer. *Hum Pathol* 2001;32:113-18.

7. Honma N, Horii R, Iwase T, Saji S, Younes M, Takubo K, Matsuura M, Ito Y, Akiyama F, Sakamoto G. Clinical importance of estrogen receptor - beta evaluation in breast cancer patients treated with adjuvant tamoxifen therapy. *J Clin Oncol* 2008;26:3727-34.

8. Horvath LG, Henshall SM, Lee C, Head D, Quinn D, Makela S. Frequent loss of Estrogen Receptor-b expression in Prostate Cancer. *Cancer Res* 2001;61:5331-35.

9. Gabal SM, Habib FM, Helmy DO, Ibrahim MF. Expression of Estrogen Receptor-β (ER-β) in Bening and Malignant Prostatic Epithelial Cells and its Correlation with the Clinico-Pathological Features. *Journal of the Egyptian Nat Cancer Inst* 2007;19:239-48.

10. Grover SK, Agarwal S, Gupta S, Wadhwa N, Sharma N. Expression of Estrogen Receptor β and Ki 67 in Benign & Malignant human prostate lesion by immunohistochemistry. *Pathol Oncol Res* 2015; 21:651-57.

11. Fixemer T, Remberger K, Bonkhoff H. Differential expression of the estrogen receptor beta (ERb) in human prostate tissue, premalignant changes, and in primary, metastatic, and recurrent prostatic adenocarcinoma. *The Prostate* 2003;54:79–87.

12. Levakov AF, Kacanski MM, Vuckovic N, Zivojinov M, Amidzic J, Sabo JI. The expression and localization of estrogen receptor beta in hyperplastic and neoplastic prostate lesions. *Vojnosanit Pregl* 2015;72:906–13.

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