

Expression of p63 Protein to Differentiate Benign Prostatic Hyperplasia and Carcinoma of Prostate in Pakistani Population

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Abstract

Background: Prostate cancer is the world's foremost and second cause of cancer associated death typically in males after lung cancer. Histopathological diagnosis of prostatic hyperplasia and prostatic carcinoma can be challenging. The expression of p63 can be used in diagnosis and differentiation of benign pro-static hyperplasia from carcinoma of prostate.

Materials and Methods: We studied sixty prostatic biopsies obtained by TURP and radical prostatectomy. For each case, clinical data was collected. The tissue sections were then diagnosed on basis of routine hematoxylin and eosin. Then immunohistochemical (IHC) analysis was performed on routinely processed, formalin-fixed, paraffin embedded tissue. We also analyzed

P63 expression in regions of benign prostatic hyperplasia and prostatic adenocarcinoma. Moreover, detailed examination of tissue sections was observed with light microscopy.

Results: Mean age of patients with adenocarcinoma was 70 ± 12 years however mean age in prostate hyperplasia cases was 66 ± 8 years. With the use of Receiver Operative Characteristic Curve (ROC), the optimal cut point found of PSA was 30.0ng/ml. The results showed that prostate specific antigen (PSA) at this cutoff had a sensitivity of 71.4%, with specificity of 74.4% and accuracy of 73.3%. So PSA could not be considered reliable independently for the diagnosis of carcinoma of prostate. Benign cases in the present study were exclusively positive for immunohistochemical expression of p63 while all the cases of prostatic carcinomas expressed negative pattern of staining.

Conclusion: Immunostaining with p63 is useful to differentiate benign prostatic hyperplasia from prostatic carcinoma, so it may be used as valuable tool in the diagnosis of prostatic carcinoma.

Keywords: p63, prostate carcinoma, benign prostatic hyperplasia, prostate specific antigen (PSA).

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Introduction

Prostatic hyperplasia is common disorder characterized by enlargement of prostate.^{1,2} It exhibits increase in number of cells of epithelium and prostrate stroma, thus forming large and fairly discrete nodules.³ It involves transitional and periurethral zones of prostate glands and is most progressive manifestation of elderly men above 50 years of age.¹ Hyperplasia of prostate subsequently leads to the problems of lower urinary tract (outflow) symptoms in males. Anatomic bladder outlet obstruction in men results most commonly over 70 yrs.^{4,5}

Prostate cancer frequency ratio is growing worldwide.⁶ Age related incidence rates of prostate cancer are diverse in different parts of world.² In Asia, the frequency is estimated three to eleven per 100,000 persons in one year, whereas incidence among African – Americans is maximum approximately 185.4 per 100,000 persons per year.⁷ In Karachi, Pakistan incidence and trend of prostate cancer were reviewed from 1995 to 1997 and 1998 to 2002. Cancer of prostate was established in roughly 3.4 percent of males from year 1995 till 1997, and graded eighth with ASR (Age standardized incidence) rate per 100,000 of 6.0 and 2.8 respectively.⁸ During the period of 1998 – 2002, prostate cancer accounted for 4.6 percent, and ranked fourth with an ASR world and CIR per 100,000 of 10.1 and 4.4. Approximately there was a 60% increase observed from 1995 and 2002. Incidence of prostate cancer in Pakistan is 3 – 7 per 100,000 persons/years from 1994 to 2004.⁹

The discovery of p63 as basal cell marker makes it a beneficial stain in challenging cases to differentiate prostatic hyperplasia from prostatic carcinoma.^{9,10} Immuno-histochemically nuclear protein p63 is diagnostically consistent in authenticating basal cells.^{2,10} In prostate needle biopsies, radical prostatectomy and transurethral resection of prostate (TURP) specimens. The protein (p63) has been identified as an important homologue of the tumor suppressor protein.¹¹ It has an important role in regulating growth and development of the urogenital system, epithelium of the salivary gland, skin, cervix, breast and particularly prostate.¹² It has an imperative role in regulation of differentiation programs and epithelial proliferation.^{11,12}

In 2012 Chastain and Oliva examined p63 as an additional indicator especially used for basal cells with High Molecular Weight-Cytokeratin (HMW-CK).¹³ HMW-CK antigen shows absence of basal cell staining due to effect of prolonged formalin fixation which results in patchy staining/absent basal cells in benign glands.⁶ Currently antibodies of p63 are established more sensitive as compared to HMW-CK for basal cells identification in prostate.² Specificity, sensitivity, and positive predictive value for p63 and HMW-CK have been considered for detection of basal cells in benign prostate glands.⁹ The specificity for both markers, was 100 percent and the sensitivity of p63 and HMW-CK were 88.37 percent and 96.70 percent correspondingly.¹³ A study reported the effectiveness of immunohistochemical investigation by p63 (basal cell specific marker) and confirmed prostatic cancer by negative staining pattern.⁶ So, p63 immunostaining is

reliable in diagnosing basal cells in benign lesions and does not give false positive result in prostatic biopsies.⁸ In local population, expression of P63 and its potential use for diagnosis of prostate hyperplasia has not been reported. Present study was designed to investigate the expression of p63 in benign prostatic hyperplasia versus prostatic carcinoma. This may help to improve early detection of hyperplasia, improve clinical practice and decrease preventable surgical procedures and morbidities.

Materials and Methods

Study Duration: The duration of this study was from 03-07-2013 to 31-12-2013.

Sample Size: Sample size of 60 patients was taken in this study.

Sampling Technique: Non-Probability, Purposive Sampling.

Sample Selection

Inclusion Criteria

1. Prostate specimens obtained either by Trans Urethral Resection prostate (TURP) or Radical prostatectomy.
2. Prostate specimens of male patients over age 40 years.
3. Patients whose data of PSA level is available.
4. Patients who give consent.

Exclusion Criteria

1. Scanty or inadequate biopsy material.
2. Improperly preserved or processed tissue.
3. Patients receiving / already received chemotherapy, radiotherapy or hormone therapy.
4. Recurrent cases of prostate cancer.

Acquisition of Specimens

After informed consent biopsy specimens of 60 patients undergoing various diagnostic and/or surgical resection procedures for hyperplasia and carcinoma of the prostate were acquired from Urology Ward of Lahore General Hospital, Lahore. Approval of study was taken from ethical committee General hospital, Lahore. Surgical tissues were obtained by radical prostatectomy specimens, transurethral resection of prostate (TURP) and ultra sound guided (USG) guided

needle core biopsies. All specimens were placed in adequate amount of 10% formalin solution.

Clinical Details

Socio-demographic information (name, age, full address, history of frequency and urgency of micturition, family history of any cancer and prostate cancer) were obtained along with data of PSA level. All the information was recorded on specially designed proforma. In hyperplasia of the prostate, predominant persistent brown nuclear staining was present and all the carcinomas of the prostate showed negative staining for p63.

The data was entered and analyzed by SPSS version 20. Data for age, size of prostate and PSA levels was described by using frequency distribution as well as Mean \pm SD. Results of p63 immunostaining expression and clinical symptoms were all described by using frequency and percentage. The comparison of age, PSA level, all clinical symptoms and p63 results between adenocarcinoma and hyperplasia groups were made by using Chi-square test. ROC curve was used to see the reliability of PSA level as a diagnostic tool against p63. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of PSA were presented by using percentage with 95% confidence interval. A p-value \leq 0.05 was considered as statistically significant.

Results

Sixty cases of benign prostatic hyperplasia and carcinoma of prostate were included in study. The average age of patients was 67 + 10 years in range from 35 – 95 years. Three (5.0 percent) cases had age lower than 50 years and twelve cases (20.0 percent) were between 51 – 60 years of age. Age of most patients ranged from 61 – 80 years with exclusion of one patient whose age was above ninety years. Average PSA levels noted for sixty cases was 46.1 \pm 91.9 ng/ml ranging from 4.0 – 625.0. Lesions having PSA level \leq 15.0 ng/ml were twenty two (36.7 percent). PSA levels more than 75 ng/ml was merely in five cases (8.3percent) and most of cases 27 (45.0%) had levels ranging between 15 and 45 ng/ml. Most (60.0%) of the cases had weight of prostate between 10 and 20 grams, with 11 (18.3%) cases between 5 and 10 grams. There were 5(8.3%) cases below 5 gm and 3 (5.0%) had weight of prostate above 30 grams with maximum weight 60.0 grams.

The mean weight recorded for the cases was 16.1 \pm 9.4g.

Among these cases 16 (26.7%) had no micturition frequency, 14 (23.3%) had mild while 20 (33.3%) and 10 (16.7%) had moderate and severe frequency of micturition. Similarly the history of urine retention was absent in 11 (18.3%) cases. Mild retention history was found in 22 (36.7%) cases with 18 (30.0%) and 9 (15.0%) cases had history of moderate and severe retention. Among other clinical symptoms burning was the major issue found in 35 (58.3%) cases while 15 (25.0%) cases had hematuria and 3 (5.0%) cases did not have any other symptom present. Most of the prostatic biopsies were TURP and only 3 were RADICAL type. On H&E examination 21 (35.0%) cases were diagnosed with adenocarcinoma and remaining 39 (65.0%) with hyperplasia of prostate. When these cases were analyzed with P63 immunostaining it was noted that 21 cases with adenocarcinoma had negative pattern on P63 stains and 39 cases of hyperplasia were positive showing persistent brown nuclear staining. Sensitivity and specificity of P63 was observed 100.0%. In comparison of cases of carcinoma and hyperplasia of the prostate, age distribution with p-value 0.227 was not significantly different. The only exception found that is insignificant, carcinoma beyond 80 years of age was noted in three cases although no case of hyperplasia was identified at this age. The PSA level was significantly different between two groups with p-value 0.002. The mean value for adenocarcinoma group was 91.6 \pm 145.2 and that for the hyperplasia was 21.5 \pm 15.3. In hyperplasia group almost 75.0% cases had PSA levels below 30 ng/ml while in carcinoma cases 33.3% had between 30 and 45 and 23.8% cases had above 75.0 ng/ml.

The weight of prostate was not seen to be significantly different between cases in two groups. The distribution was same with average weight of 14.2 \pm 7.4 and 17.1 \pm 10.2 grams in adenocarcinoma and hyperplasia groups respectively with p-value 0.628.

On comparison it was noted that the micturition frequency was not present in most (76.2%) of adenocarcinoma cases, but to some extent this symptom was present in all cases of prostatic hyperplasia so the p-value noted here was $<$ 0.00. In the same manner the adenocarcinoma cases either had no history of retention of urine (47.6% cases) or showed history of very considerable retention (47.6% cases). Whereas in case of hyperplasia of prostate single patient complaint of retention whereas 67% complained of moderate to

severe retention of urine. Difference was proved to be significant again with p-value < 0.001.

Among other clinical symptoms, burning micturition was the major issue present only in hyperplasia cases and was present in 89.7% cases. Hematuria was the problem found only in adenocarcinoma cases and found in 71.4% of cases. This difference was highly significant between two groups with p-value < 0.001. In the end when cutoff of PSA was searched by receiver operative characteristic curve (ROC), the optimal point found was 30.0 ng/ml. The diagnosis showed that PSA at this cut off had a sensitivity of 71.4%, with specificity of 74.4% and accuracy of 73.3%. So could not be considered reliable independently.

Table 1: Reliability of PSA level as diagnostic tool for prostatic carcinoma on the basis of diagnostic measures.

Measure	Value	95% Confidence Interval
Sensitivity	71.4	60.0 – 82.8
Specificity	74.4	63.4 – 85.4
Positive Predictive Value	60.0	47.6 – 72.4
Negative Predictive Value	82.9	73.4 – 92.4
Accuracy	73.3	62.1 – 84.5

Discussion

For screening of prostate cancer, PSA level was considered the screening tool since mid-1980s. Currently annual digital rectal examination (DRE) and serum PSA level determination are recommended screening measures for carcinoma of prostate. The normal PSA value is considered to be 4.0 ng/ml, the borderline value ranges from 4 – 10 ng/ml while higher than 10 ng/ml was believed to be high level. Cases with PSA value more than 4ng/ml despite DRE results, biopsy is required to reach the final diagnosis. Highest specificity was considered to be maximum in males omitted without prostatic cancer whereas highest sensitivity was considered to be maximum number of prostatic cancer findings. Highest specificity and highest sensitivity are present at cut off value of 4.0ng/ml.¹⁴ PSA is highly expressed in both benign prostatic epithelia and prostate cancer cells.¹⁵

Basic hematoxylin and eosin (H&E) staining criteria are used for morphological assessment of benign

and carcinoma cases of prostate.¹⁶ Malignancy features are diagnosed easily on the basis of infiltrative glands lined by luminal cells with increase in nucleolar and nuclear size and absence of basal cells.¹⁷ Numerous auxiliary techniques have been suggested for the recognition of malignancy. The role of immunohistochemistry is very important to differentiate the lesions.¹⁸

For this study, average PSA level for 60 cases was 46.1 ± 91.9 ng/ml ranging from 4 to 625. Twenty-two (36.7 percent) cases had PSA level less than 15.0 ng/ml. Merely five (8.3percent) cases had PSA level more than 75ng/ml beside most of twenty-seven(45 percent) cases had the level from 15 to 45ng/ml. PSA level for both lesions hyperplasia and carcinoma were also compared. Difference remained significant (p = 0.002) statistically. Results found here are in accordance with the findings of study by Cabarkapa et al.¹⁴ Frequency of micturition was a common clinical symptom, absent in maximum (76.2 percent) cases of prostate adenocarcinoma, whereas hyperplasia cases complaint of frequency to certain amount. p-value observed was less than 0.001. In the same manner, the adenocarcinoma cases had no symptom of urinary retention or very mild retention of urine, whereas in hyperplasia of prostate, merely single case didn't show symptoms of retention and 67 percent show moderate and severe retention. This difference was once more significant with p-value less than 0.001. In adenocarcinoma cases hematuria was the symptom established in (71.4 percent) cases. Our results are similar with study conducted in 2008 by Khan.¹⁹

In this study, identified on the basis of eosin and hematoxylin staining, 21 cases of prostatic carcinoma whereas 39 cases of prostate hyperplasia were stained with monoclonal antibody (p63) by standard heat-induced retrieval as well as avidin-biotin method. Hyperplasia and carcinoma of the prostate showed expression with p63. It was established that p63 did not exist in any case of carcinoma however 39 cases of prostatic hyperplasia exhibited positive, nuclear staining pattern. Our results are in accordance with the study in which they observed that there were persistent basal cells staining with p63, of benign and atrophic lesions, and complete lack of basal cells when stained with p63 in prostatic carcinoma.¹⁰

In a recent study Popkov VM et al (2013) comparatively assessed the molecular markers and concluded a high histochemical score of P63 in a group of patients having prostatic hyperplasia (Hs = 91) and prostatitis (Hs = 83) where some basal cells are intact for

the positive expression of the P63 and very low score for the carcinoma of the prostate (Hs = 17).¹⁸

A study showed the effects of p63 deficiency in the development of the epithelial organs, namely the agenesis of the squamous epithelia, mammary, Salivary, and lacrimal glands. It was demonstrated a very weak expression of the p63 in a series of 130 prostatic carcinomas and in prostate cancer cell line.²⁰ In another study a case of atypical adenomatous hyperplasia or adenosis of the prostate showed positive staining of the patchy basal cells and highlighted the immunostaining with p63 in the accurate diagnosis of the prostatic adenosis, a benign mimicker of prostate adenocarcinoma.²¹ These outcomes are also similar with Rashed who conducted comprehensive investigation in 2012. Fifty needle biopsy samples with p63 expression, comprising thirty with small foci of prostatic adenocarcinomas and twenty benign prostates (< 1mm or < 5% of needle core tissue) were stained by p63.²² Outcomes of thirty cases of small foci of prostatic adenocarcinomas remained negative for basal cell staining p63 (nuclear stain). p63 positivity was found in all the benign glands of prostate and they were recognized markedly. So it was established from present study that p63 might aid in diagnostic precision and this assisted to avoid taking new biopsies in finding small foci of carcinoma of prostate.²³

Current study similarly exhibited comparable findings with other studies where p63 responded with basal cells in ninety eight percent cases (254 out of 259 cases showed positive result). The concentration of nuclear staining wasn't dependent on the techniques of fixation. In atrophic and benign lesions, ten to hundred percent persistent basal cell staining was noted with p63. All cases of prostatic carcinoma remained negative for p63.²⁴

Conclusion

In this study, all benign cases were positive for immunohistochemical expression of P63 while all cases of carcinoma expressed negative staining pattern with p63. Immunostaining with p63 escalates the diagnostic yield, consequently it may be used as a valuable tool in differential diagnosis of prostatic carcinoma.

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