Original Research Article

Immuno-Histochemical Study of p63 Expression in Prostatic Lesions

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Abstract

Introduction: Diseases of Prostate gland are responsible for significant morbidity and mortality among adult males all over the world. This study is carried out to study the usefulness of immuno-histochemistry using p63 basal-cell marker in prostatic lesions, especially the morphologically ambiguous ones. **Aims:** i) To study histopathological features of prostatic lesions. ii) To study expression of p63 basal-cell marker in prostatic lesions. iii) To correlate p63 expression with histopathology. **Materials and methods**: A descriptive observational study was conducted at Mahadevappa Rampure Medical College, Kalaburagi, Karnataka, India from 1st July 2017 to 30th June 2020. Specimens were sent for routine histopathological analysis followed by immuno-histochemistry analysis with p63. **Statistical analysis used**: Chi Square test was used for qualitative data analysis and Receiver Operator Curve was used for dichotomous data. Mean with Standard Deviation was used for quantitative data analysis. The results were considered statistically significant when *p* value was <0.05. **Results**: Of 52 specimens, the age of patients ranged from 60 to 88 years and mean being 68.61 years. Majority of patients presented with complaint of overflow incontinence. We encountered 36 cases of Benign Prostatic Hyperplasia, 13 cases of Prostatic Adenocarcinoma & 3 cases of Prostatic Intra-Epithelial Neoplasia. The ability of p63 to distinguish between Prostatic Carcinoma and non-Carcinomatous Prostatic lesions was statistically significant. Diagnostic Accuracy of p63 was 96.15%. **Conclusion**: Ability of p63 to distinguish between Prostatic Carcinoma and non-Carcinomatous Prostatic lesions was statistically significant. It helps in distinguishing morphologically ambiguous lesions of the prostate into benign or malignant lesions.

Keywords: Adenocarcinoma, Benign Hyperplasia, Intraepithelial Neoplasia, p63, Prostate.

Introduction

Diseases of Prostate gland are responsible for significant morbidity and mortality among adult males all over the world. The three main pathologic processes that affect the prostatic gland are inflammation, nodular hyperplasia (NH) and tumors. Prostatic carcinoma is globally the second most frequently diagnosed cancer and the sixth leading cause of cancer death in males. Based on GLOBOCAN 2018 estimates, 7.1% of all cancers in men were prostate cancers. In India, it constitutes about 5% of all male cancers[1,2].

The diagnosis of prostatic adenocarcinoma is usually readily made on morphological grounds. However, it is difficult to distinguish from 'atypical small acinar proliferation, (ASAP)' or 'Morphologically Ambiguous Lesion of Prostate', including atrophy, atypical adenomatous hyperplasia, basal cell hyperplasia and high-grade prostatic intraepithelial neoplasia (PIN). Also recognizing small foci of cancer in needle biopsies can be difficult without an adjuvant diagnostic marker[3]. In such situation immunohistochemistry can be a useful adjuvant for establishing a definitive diagnosis of such prostatic lesions[3]. The present study is carried out to study the utility of immuno-histochemistry using p63 basal-cell marker in prostatic lesions and to correlate its expression with histopathological study.

Materials & methods

A 3-year descriptive observational study was carried out from 1st July 2017 to 30th June 2020 on 52 prostatic lesions received for routine histopathological examinations from Basaveshwar Teaching and General Hospital attached to Mahadevappa Rampure Medical College, Kalaburagi and various private hospitals and laboratories in and around Kalaburagi. The study was approved by the institutional ethics committee of Mahadevappa Rampure Medical College, Kalaburagi on 29/10/2018.

All types of surgical specimens operated for prostatic lesions were collected, routinely processed. H&E sections were studied and immuno-histochemistry was carried out.

For p63 immunostaining, sections were de-paraffinized, rehydrated, and subjected to microwaving in citrate or EDTA buffer (as per vendor specifications and product instruction manual, including microwaving wattage and duration). Slides were allowed to cool at room temperature for 30 minutes. The p63 4A4 mouse monoclonal antibody (1:50 dilution) from AS Bioscience was applied at room temperature. Peroxidase activity was localized using 3,3-diaminobenzidine or 3,3-diaminobenzidine-nickel chloride. Standardized development time periods allowed accurate comparison of all samples.

Criteria for assessing p63 staining as optimal includes moderate to strong, distinct nuclear staining reaction in basal cells in the prostate glands is observed.

Inclusion Criteria

All needle biopsies, transurethral resections and prostatectomy specimens of all ages were included in the present study.

Exclusion Criteria

Extensive tumour necrosis of the specimen were excluded.

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Statistical Analysis was done by using the software SPSS (Statistical Package for the Social Sciences) version 20.0. Chi Square test was used for qualitative data analysis and Receiver Operator Curve was used for dichotomous data. Mean with Standard Deviation was used for quantitative data analysis, wherever necessary. The results were considered as statistically significant when the *p* value was <0.05.

Results

A total of 52 specimens were received and included in our study as per the aforementioned inclusion and exclusion criteria. The Age-wise distribution shows that most cases encountered (25 cases, 48.07%) were in the 61-70 years age group (see **Graph 1**). The larger number of patients (25 cases, 48.09%) presented with the complaint of overflow incontinence. The patients who presented with haematuria were cases of bilateral nephropathy with prostatomegaly or vesicle calculus or cystitis with prostatomegaly and some presented with pain in abdomen/groin were cases of inguinal hernia with prostatomegaly.

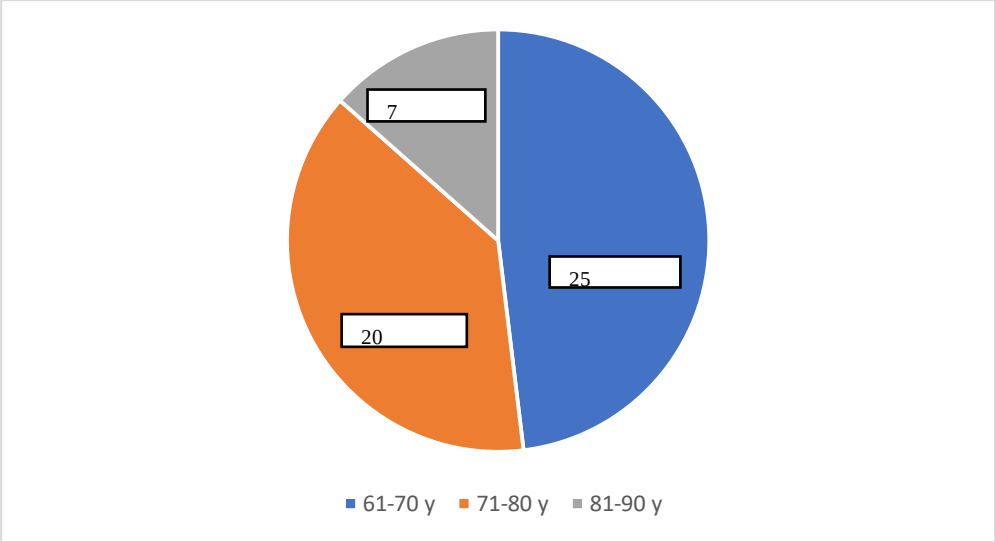

Fig. 1: Age distribution

Most number of specimens received were trans-urethral resection amounting to 42 (80.76%), open prostatectomy cases were 9(17.3%) and prostate biopsy (TRUS guided) was 1 (1.94%). There was a total of 36 benign prostatic hyperplasia cases, 3 prostatic intraepithelial neoplasia cases and 13 prostatic adenocarcinoma cases. Most of the prostatic adenocarcinoma cases were Gleason Grade 4 cases (5 out of 13 cases, 38.46%); as per 2014 ISUP Consensus Gleason Scoring and Grading System[4].

The Age-wise distribution shows that the majority of Prostatic Adenocarcinoma cases (7 out of 25 cases, 53.84%) occurred in the 61-70 years age group. Chi-Square analysis for statistical significance gave Chi-square value of 1.460, and *p*-value was 0.8338, which is >0.05. Therefore, there was no statistically significant difference of age of incidence between patients of BPH vs. PIN vs. Prostatic Adenocarcinoma.

Of the 13 Prostatic Adenocarcinoma cases, 1 was Pseudo-hyperplastic, 2 were of the Foamy-gland variant, 2 were each of atrophic variant and microcystic variant. However**,** the most common type was the Conventional Gleason pattern 4 (cribriform glands) Prostatic Adenocarcinoma with 6 cases.

Majority of the BPH Cases showed PSA levels (**Table-1**) to be below 4 ng/mlL(31 out of 36 cases, 86.11%); PIN Cases showed values of 3.8, 8.9 & 31.83 ng/ml respectively. The Carcinoma cases showed PSA values in the 7.1-14 ng/mL range (6 out of 13 cases, 46.15%). However, majority of the cases showing PSA >21 ng/ml were carcinomas (3 cases, 75%). Chi-Square analysis for statistical significance gave Chi-square value of 37.62, and p-value was <0.0001; which is <0.05. Therefore, there was a statistically significant difference of PSA levels between BPH, PIN & Prostatic adenocarcinoma cases.

Table-1: Correlation of cases with their respective PSA levels (*n*=52)

PSA Value Range	No. of BPH (±prostatitis) Cases	No. of PIN Cases	No. of Prostatic Adenocarcinoma Cases	Total	Percentage
0-4 ng/ml	31	1	0	32	61.56%
4.1-7 ng/ml	3	0	2	5	9.61%
7.1-14 ng/ml	2	1	6	9	17.30%
14-21 ng/ml	0	0	2	2	3.84%
>21 ng/ml	0	1	3	4	7.69%

Total	36	3	13	52	
Percentage	69.24%	5.76%	25%		100%

Out of the 39 cases which had intact basal cell layer (as per Histopathology of BPH & PIN), 37 cases were p63-positive (**Figure 1**). However, all of the cases with absent basal cells (as per histopathology of prostatic carcinoma) were p63-negative (**Table-2**). Thus, the ability of p63 to distinguish between Prostatic Carcinoma and non-Carcinomatous Prostatic lesions is statistically significant. On statistical analysis the sensitivity was 94.87% and specificity was 100%. The positive predictive value, negative predictive value, diagnostic accuracy was 100%, 86.67% and 96.15% respectively.

Table 2: **Correlation of Histopathological diagnosis with Immuno-Histochemical Staining profile(*n*=52)**

Histopathological diagnosis Immuno-Histochemical Staining profile	Benign Adenoleiomyomatous Hyperplasia of Prostate (±prostatitis) & Prostatic Intraepithelial Neoplasia	Prostatic Adenocarcinoma	Total	Percentage
p63 positive	37	0	37	71.16%
p63 negative	2	13	15	28.84%
Total	39	13	52	
Percentage	75%	25%		100%

Out of the 37 p63-positive cases, a majority had PSA-values <4ng/mL (30 out of 37 cases; 81.08%), whereas out of the 15 p63-negative cases, majority showed PSA-values in the 7.1-14ng/mL range (6 out of 15 cases; 40%). In order to determine an appropriate cut-off value for PSA levels considering p63 staining profiles, a Receiver Operator Curve (ROC) was plotted by considering each PSA-value data as a cut-off and calculating sensitivity & specificity for them and plotting them on a sensitivity% vs. 100-specificity% graph. The Area under Curve for the ROC was 0.9467, indicating "outstanding" ability to distinguish p63 negative (Carcinoma) cases from p63 positive (non-Carcinoma) cases; *p*-value was <0.0001 .The ROC and the sensitivity & specificity table (for each PSA cut-off value), both indicate that <5.4ng/mL was an appropriate cut-off value to distinguish p63 positive cases from p63 negative cases.

Discussion

In the present study, we came across 52 prostatic lesions in our tertiary care hospital and other private hospitals & diagnostic laboratories in & around Kalaburagi, Karnataka. The demographic & clinico-pathological data were collected to analyze any association of age, clinical presentation, PSA-levels & p63-IHC to the actual histopathological diagnosis. In the present study, the age of the patients ranged from 60 to 88 years, and the mean age was 68.61 ± 7.74 years. We note that in the present study majority of cases were in the 61-70 years age-group which was also seen in many previous studies done by Kumar K.P.V, Palsdottir et al and Ranjitham et al[7,9,15].

The majority of cases was of BPH (69.23%) which was concordant to other studies done by Josephine et al, Javed et al and Anand et al (73.58%, 47.77%, 72.9%). The studies by Josephine et al and Anand et al showed cases of PCa to be 2nd-most frequent diagnosis (18.86% & 14.2% respectively), with PIN cases being the least frequ diagnosis (7.54% & 12.9% respectively)[5,6,10]. The present st showed similar trend with PCa being the 2nd-most frequent diagnosis (25%) and PIN cases being least frequent (5.76%). Majority of the BPH cases were in the 61-70 years age-group in our study and similar trend was seen in all the previous studies (53.84%, 51.28%, 55.8% & 35.33% respectively). The previous studies also showed that majority of PCa cases occurred in the 61-70 years age-group (50%, 63.6% & 42.85% respectively)[5,6,7]. The present study also showed a similar trend (Majority of PCa cases in 61-70 years age group; 17 cases; 47.22%). However, inspite of noting these trends in the age distribution, none of these studies (including the present study) could establish any statistically significant difference of age of incidence between BHP, PIN & PCa cases by using a test of significance.

Serum Prostate Specific Antigen is a non-specific marker for prostatic lesions but is still used as one of the most important diagnostic modalities as almost any phenomenon inside the prostate, such as hyperplasia, inflammation, tumors, may lead to the increase of serum PSA value. The increase in PSA values also depends on upon the differentiation of tumor cells[11].

The present study showed majority of PSA values amongst PCa cases to be within 7.1-14ng/mL range. Two of the three previous studies showed majority of PSA values amongst PCa cases to be >21ng/mL; the study from Sweden by Palsdottir *et al* showed majority of PCa cases to have PSA values between 0-4 ng/Ml[6,9,11]. However, the present study demonstrates highly significant statistical difference of PSA-values between BPH, PIN & PCa cases, similar to what was seen in the studies done by Javed et al[6] and Banerjee et al[11].

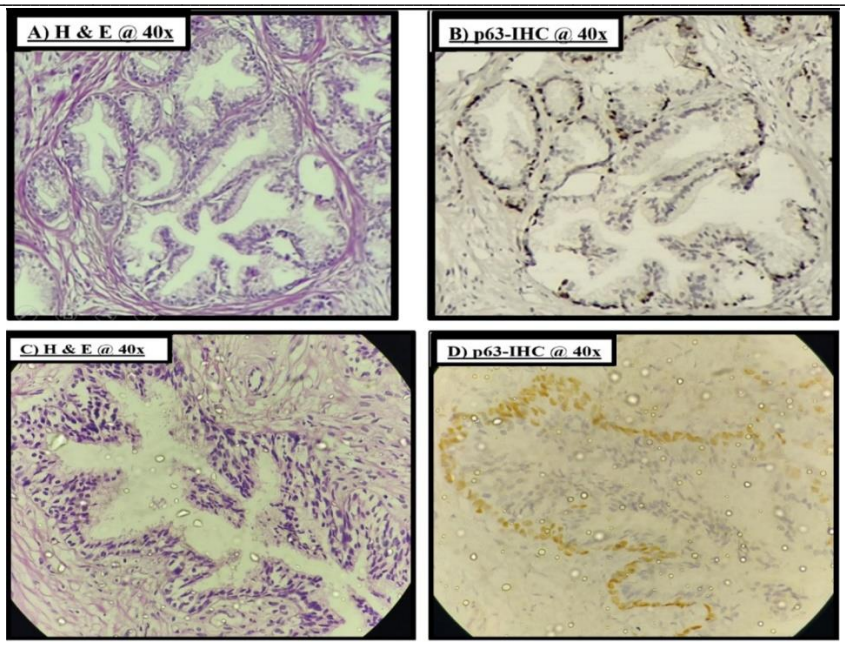

Fig. 2: Benign Nodular Hyperplasia of prostate showing glandular hyperplasia (A); same area under p63 where nuclei of basal cells are showing positive reaction (B). Prostatic Intra-Epithelial Neoplasia (C) with same area under p63 showing intact basal cell positivity for p63 staining (D).

The most important part of the present study, however, was to establish diagnostic accuracy of p63 Immuno-histochemical stain when compared to histopathological diagnosis; based on its ability to stain basal cells and therefore stain BPH & PIN cases as p63-positive (**Figure 2**) and PCa cases as p63-negative (**Figure 3 & 4)**. The present study and all the previous studies analysed had statistically significant differences in p63 staining profile of BPH vs. PIN vs. PCa cases (**Table 3)**[12,13]. Basal epithelial cells are stained with p63 in normal, benign hyperplasia, and intraepithelial neoplasm whereas a very high percentage of adenocarcinomas (90%) react negatively with p63[14].

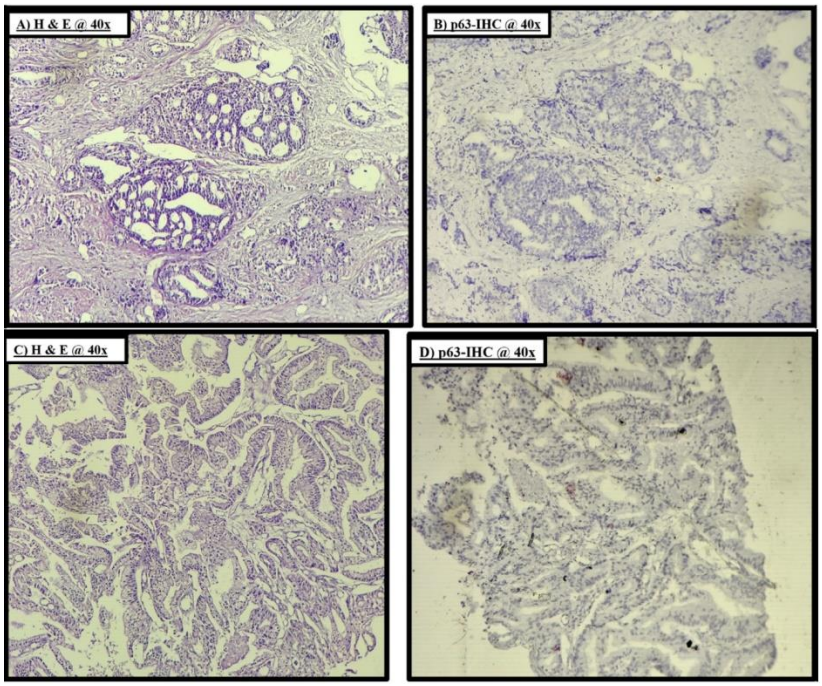

Fig. 3: Gleason pattern 4 glands of Prostatic Adenocarcinoma. A) showing typical "cribriform" Gleason Pattern 4 glands; B) showing same focus under p63-IHC with p63-negative glands. Prostatic Adenocarcinoma - Pseudo-Hyperplastic variant (Gleason pattern 3 glands), C) showing glands resembling benign hyperplastic glands at the architectural level, including papillary infoldings and gland branching, but having high N:C ratio, D) showing p63-negative glands under immuno-histochemistry

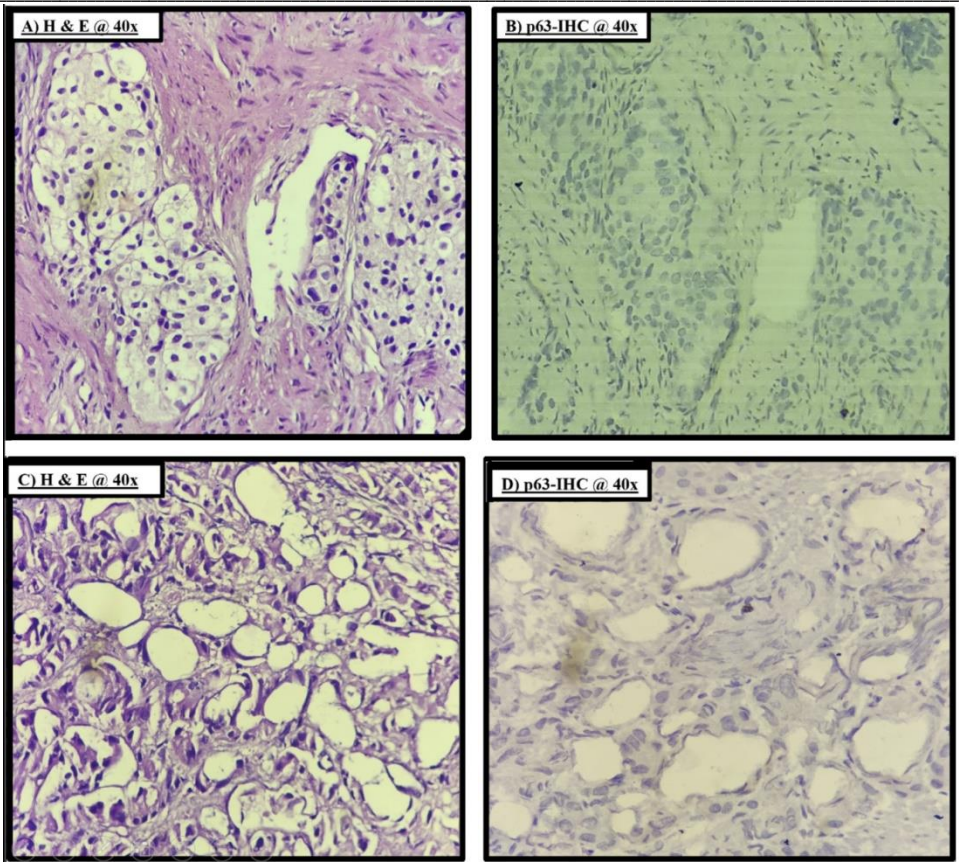

Fig. 4: **Prostatic Adenocarcinoma - Foamy Gland variant (Gleason pattern 3 glands). A) shows foamy ("xanthomatous") glands because of the massive accumulation of lipids B) showing p63-negative glands. Prostatic Adenocarcinoma - Atrophic variant (Gleason pattern 3 glands). C) shows tumor cells with an attenuated cytoplasm, such that the nuclei occupy almost the entire cell height D) showing p63-negative glands**

Table 3: Table comparing p63 staining characteristics encountered in previous studies compared to present study

Age	Samundeeswari et al[12],(*n*=12)				Rathod et al[13],(*n*=80)				Present Study(*n*=52)			
	BPH	PIN	PCa	Total	BPH	PIN	PCa	Total	BPH	PIN	PCa	Total
p63 positive	4	3	0	7	40	0	0	40	34	3	0	37
p63 negative	0	1	4	5	0	0	40	40	2	0	13	15
Total	4	4	4	12	40	0	40	80	36	3	13	52
Statistical Significance	YES Highly Significant p<0.05				YES Highly Significant p<0.05				YES Highly Significant p<0.05			

Another important aspect of the present study was to analyse and establish any association between PSA-levels and p63-staining profile as per patient's Histopathological Diagnosis. Tabulation showed that out of the 37 p63-positive cases, a majority had PSA-values <4ng/mL (30 out of 37 cases; 81.08%), whereas out of the 15 p63-negative cases, majority showed PSA-values in the 7.1-14ng/mL range (6 out of 15 cases; 40%). To study and establish any association between PSA-levels and p63-staining profile as per patient's histopathological diagnosis, we plotted a Receiver Operator Curve. The ROC and the sensitivity & specificity table (for each PSA cut-off value), both indicate that <5.4ng/mL is an appropriate cut-off value to distinguish p63 positive cases from p63 negative cases.

Conclusion

p63-Immuno-Histochemistry is an emerging adjuvant staining modality to help distinguish morphologically ambiguous lesions of the prostate into benign or malignant lesions. The present study found a statistically significant difference in p63-staining profile of BPH, PIN, PCa cases. Serum Prostate Specific Antigen is considered a non-specific marker for prostatic lesions but is still used as one of the most important diagnostic modalities for Prostate Cancer. The present study showed a statistically significant difference in PSA-values between BPH, PIN & PCa cases. Furthermore, the present study found PSA-value to have a statistically significant difference among p63-positive vs. p63-negative cases.

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