Section: Miscellaneous

Original Research Article

HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL EVALUATION OF PROSTATIC LESIONS: A CROSS-SECTIONAL STUDY

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Received : *23/11/2024*
Received in revised form : *11/01/2025*
Accepted : *28/01/2025*

Keywords:
Prostatic lesions, Benign prostatic hyperplasia, Prostate carcinoma, Histopathology, Immunohistochemistry, p53, Ki-67.

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DOI: 10.47009/jamp.2025.7.1.46

Source of Support: Nil,
Conflict of Interest: None declared

Int J Acad Med Pharm
2025; 7 (1); 232-237

Abstract

Background: Prostate gland lesions, including benign prostatic hyperplasia (BPH), prostatitis, and carcinoma, are prevalent among aging males, contributing significantly to morbidity and mortality. While histopathological examination remains the gold standard for diagnosing prostate carcinoma, mimickers such as benign hyperplasia and inflammatory conditions pose challenges. To evaluate the histopathological spectrum of prostatic lesions and the immunohistochemical expression of p53 and Ki-67 in prostatic carcinoma to identify prognostic markers. **Materials and Methods:** A cross-sectional study was conducted in the Department of Pathology at B.R.D. Medical College, Gorakhpur, over one year (July 2019–July 2020). Prostatic biopsies, including transurethral resection of the prostate (TURP) and radical prostatectomy specimens, were collected. Specimens were processed using routine histopathological techniques, stained with hematoxylin and eosin, and analyzed. Immunohistochemical staining for p53 and Ki-67 was performed. Data were statistically analyzed using Fisher's exact test, with $p < 0.05$ considered significant. **Result:** Patients ranged from 39 to 81 years, with the highest incidence (48%) in the 70–79 age group. Common symptoms of carcinoma included urinary frequency (30%), difficulty in voiding (26.6%), and nocturia (13.3%). Among 100 cases, 60% were adenocarcinoma, 10% intraepithelial neoplasia, and 30% benign lesions. BPH accounted for 76% of benign cases. Gleason score 7 was the most common (60%), with 36 cases showing moderate differentiation. Poor differentiation was observed in 31.6% of cases. Strong nuclear positivity for p53 was significantly associated with poorly differentiated adenocarcinoma (94.7%), while well-differentiated tumors showed minimal positivity. **Conclusion:** Prostatic carcinoma predominantly presents in older males with varied histopathological patterns. Immunohistochemical markers, such as p53 and Ki-67, demonstrate significant prognostic value, aiding in the differentiation of tumor grades and guiding therapeutic strategies. Enhanced early detection and accurate grading through combined histopathological and immunohistochemical approaches are imperative for effective management of prostatic carcinoma.

INTRODUCTION

Prostate gland is one of the most commonly affected organ in male with increasing age, accounting for significant morbidity and mortality.[1] Lesions of prostate are an area of constant interest to clinician as well as pathologist. Recently, there is considerable change in the understanding of many prostatic diseases. Accurate diagnosis of prostatic disease frequently requires simultaneous clinical history, biochemistry, imaging techniques and surgical pathology laboratory.[2] Even following detailed histopathological examination of biopsy tissue, taken from precisely defined microanatomic sites within the prostate, informed opinion as to the diagnostic or prognostic significance of particular morphological features, frequently remain controversial, to the extent that the distinction of benign from malignant neoplastic diseases may not be possible.[3]

ISSN (O): 2687-5365; ISSN (P): 2753-6556

In India carcinoma of prostate occupies 2nd to 10th rank among cancer in men, in various metro cities as per national cancer registry.[4,5] Significant advances have occurred in the understanding of pre malignant epithelial lesions as well as new clinical techniques, enhancing early detection of cancer, such as transrectal ultrasound and serum levels of prostate specific antigen (PSA). There is substantial increase in number of prostate needle biopsies due to increased awareness and the wide spread use of serum PSA as a mass screening test along with imaging studies for prostate cancer. Accurate diagnosis on needle core biopsy or transurethral resection of prostate (TURP) specimen is of utmost importance because if diagnosed early for malignancy, patient is benefitted as a result of a lesser invasive procedure instead of more radical procedures that is associated with significant mortality and morbidity.[6]

However, biopsy remains the gold standard for final diagnosis. Histological diagnosis of prostatic cancer is usually based on morphological features such as growth pattern, nuclear atypia and absence of basal cells.[5] However, there are various mimickers of prostate carcinoma such as benign hyperplasia, prostatitis, atrophy, adenosis, atypical adenomatous hyperplasia and nephrogenic adenoma which makes the diagnosis of prostatic widely used and accepted histopathological method for providing information about the prognosis of prostate carcinoma. This grading system is based entirely on the histological pattern of differentiation and arrangement of section. Despite advances in screening and multimodal management of this disease, overall survival remains poor. Hence, there is a need to identify various prognostic markers for developing new therapeutic strategies for better management of Prostate carcinoma patients.

MATERIALS AND METHODS

It was a cross-sectional study conducted in Department of pathology, B.R.D Medical College, Gorakhpur, U.P for a period of 1 year (July 2019 to July 2020).

Selection of Cases- The proposed study has been carried out on patients of various prostatic lesions including benign, premalignant and malignant lesions attending surgery OPD and on admitted patients in the surgery wards of Nehru Chikitsalay, B.R.D Medical College, Gorakhpur, UP. during in a period ranging from July-2019 to July-2020. Detailed history, clinical findings specially digital rectal examination, PSA value, radiological and other investigative findings were noted. After taking informed consent, (Annexure-5) histopathological examination and immunohistochemical expression of p53 and Ki-67 were carried out on prostatic biopsies including trans urethral resection of prostate (TURP) specimens, as well as radical prostatectomy specimens, received in the department of pathology.

Inclusion Criteria

All prostatic biopsies specimens suspected to have benign and malignant prostatic lesions along with TURP specimens, TRUS biopsies, radical prostatectomy specimens received in the department of pathology

Patients who agree to sign on consent form.

Exclusion Criteria

Autolyzed sample.

Inadequate sample.

Preparation of tissue for histo-pathological examination- Prostatic biopsy tissues were fixed in 10% formalin saline and subjected to histopathological examination using paraffin embedding technique. Tissue blocks were prepared using paraffin wax of 5860 0C melting points. All the paraffin blocks were preserved for section cutting. Thin sections of 2-3 micron were cut after dewaxing and then stained by hematoxylin and eosin stain. Histopathological diagnosis was made and then freshly cut sections were also used for immunostaining.

Processing of histopathological slides:

Fixation: The tissues were subjected to overnight fixation in 10% formalin solution.

Embedding: It involved

- Removal of water by alcohol dehydration
- Infiltration of xylene as a solvent for paraffin wax.
- Paraffin wax impregnation.

Microtomy:

- Sections of 2-3µ thickness were cut from routinely processed paraffin embedded blocks and gently lowered on the surface of water bath at 45ºC.
- These sections were taken on alcohol cleaned glass slides smeared with a thin film of egg albumin.
- The slides with the sections were warmed on a hot plate at 58ºC for 1 hour, cooled and stored in a box for staining.
- Wax removal was done in xylene, slides were kept in xylene for 2 minutes and 2 such changes were done.
- Removal of xylene was done with absolute alcohol. Slides were kept in absolute alcohol for 2 minutes and 2 such changes were made.
- Treatment with descending grades of alcohol in 90% alcohol for 1 minute and in 70% alcohol for 1 minute was performed.
- Finally the sections were brought to the deionised water.

Hematoxylin and eosin staining procedure: Section were stained in a solution of Harris hematoxylin for 5-15 minutes. Section were decolorized with 1% acid alcohol for 10-20 seconds and again washed with tap water. Section were kept in warm water for 5 minutes, and counter stained with 1% aqueous eosin for 5 minutes. And then section was washed rapidly in water to remove excessive amount of eosin.

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Then it was dehydrated by several changes of increasing grade of alcohol, cleaned in xylene and mounted with Dextrin 80 Di-butyl phthalate Xylene (DPX) mount.

The predominant tumor pattern (referred to as primary) was graded from 1 to 5, and the 'secondary' pattern (if present) was graded similarly, the two numbers were added to obtain the Gleason score. For immunohistochemical staining, by antibodies against p53 and Ki-67, the kit literature of the manufacturer was followed. Strong brown nuclear immune-reactivity was considered as positive staining. The immune-quantification was performed using percentage of tumor cells that react with antibody.

Statistical Analysis: Appropriate statistical tools were adopted for the data analysis. Analysis was done by data sorting method, classified by tabulation and presentation by pie charts, and histograms. Statistical method such as calculation of mean, standard deviation, Fisher's exact test was employed to find out the significance of the study and a p-value of <0.05 was considered significant.

RESULTS

As per [Table 1] the age of patients in the study was ranged between 39 to 81years.Youngest patient was 39 years old and oldest patient was 81 years old. It was observed that maximum number of cases (48%) was in the age group of 70-79 years.

Table 1: Age wise distribution of prostatic lesions.

Age (Years)	Prostatic lesions (Number)	Percentage (%)
30-39	1	1%
40-49	3	3%
50-59	8	8%
60-69	32	32%
70-79	48	48%
≥ 80	8	8%
Total	100	100%

Table 2: Clinical presentation of Prostatic Carcinoma cases

Symptoms	Number of cases (60)	Percentage (100%)
Frequency of urine	18	30%
Nocturia	08	13.3%
Urgency of urine	04	6.6%
Straining	04	6.6%
Difficulty in voiding	16	26.6%
Hesitancy	03	5%
Incomplete voiding	02	3.3%
Hematuria	03	5%
Acute retention	01	1.6%
Poor stream of urine	01	1.6%

Most of the patients with prostatic carcinoma presented with complaint of frequency of urine (30.0%), difficulty in voiding (26.7%) and nocturia (13.3%), followed by urgency of urine and straining (both 6.6%). Hesitancy and hemtauria were observed in 5% (3 cases).

Table 3: Gleasons Score

Gleason's score	Number of cases	Percentage (%)
≤6	5	8.3%
7	36	60%
8	15	25%
9	4	6.7%
10	0	00%
Total	60	100%

Carcinoma prostate was categorized according to Gleasons score (combined Gleason grade). Gleason score 7 was the commonest pattern observed in 36 cases (60%), followed by Gleason score 8 in 15 cases (25%). Gleason score of 9 was observed in 4 cases (6.7%). Gleason score of 6 was observed in 4 cases (13.3%) and Gleason score of 4 was seen in only one case (3.3%).

Table 4: Histological differentiation of prostatic adenocarcinoma cases

Histological differentiation	Number of cases	Percentage (%)
Well differentiated adenocarcinoma	5	8.3%
Moderately differentiated adenocarcinoma	36	60%
Poorly differentiated adenocarcinoma	19	31.6%
Total	60	100%

ISSN (O): 2687-5365; ISSN (P): 2753-6556

Based on tumor differentiation, 5 cases (8.3%) were well differentiated prostatic adenocarcinoma. 36 cases (60%) were moderately differentiated adenocarcinoma and 19 cases (31.6%) were poorly differentiated adenocarcinoma.

Table 5: Distribution of prostatic lesions on the basis of histopathological diagnosis

Prostatic lesion	Number of cases	Percentage (%)
Prostatic adenocarcinoma carcinoma	60	60%
Prostatic intraepithelial neoplasia (PIN) LGPIN (Low grade) HGPIN(High grade)	10 6 4	10% 60% 40%
Benign prostatic hyperplasia	23	76%
Benign prostatic hyperplasia with acute prostatitis	1	3.3%
Benign prostatic hyperplasia with chronic prostatitis	4	13.3%
Benign prostatic hyperplasia with Granulomatous prostatitis	1	3.3%
Benign prostatic hyperplasia with sqamous metaplasia	1	3.3%
Total	100	100%

Among malignant cases, all the cases 60 cases were prostatic adenocarcinoma. Out of 10 cases of prostatic intraepithelial neoplasia, 6 cases (60%) were low grade prostatic intraepithelial neoplasia and 4 cases (40%) were high grade prostatic intraepithelial neoplasia. Out of 30 cases of benign lesions, majority of lesion were benign prostatic hyperplasia (76%), 4 cases (13.3%) of BPH were associated with chronic prostatitis and 1 case (3.3%) associated with acute prostatitis. Squamous metaplasia was seen in 1 case (3.3%).

Table 6: Frequency of p53 expression in relation to tumor differentiation and Gleason's grade of prostatic adeno carcinoma

Gleason's grade	P53 expression (%)			
	0 (0%) Number (%)	1 (<10) Number (%)	2 (10-33) Number (%)	3 (> 33) Number (%)
Well differentiated adenocarcino ma(n=5)	3(60%)	2(40%)	0(0%)	0(0%)
Moderately differentiated adenocarcino ma (n=36)	9(25%)	5(13.8%)	7(19.4%)	15(41.6%)
Poorly differentiated adenocarcino ma(n=19)	1 (5.3%)	4 (21.0%)	5(26.3%)	9 (47.4%)
Total (n=60)	13	11	12	24

In Prostatic carcinoma, 3 out of 5 (60%) well-differentiated tumors showed absence of positivity while 2 cases (40%) showed grade I positivity. 15 out of 36 (41.6%) moderately differentiated tumor revealed strong nuclear positivity with grade 3 positivity and 9 cases showed grade 2 positivity. 9 cases out of 36 (25.0%) did not express p53 positivity. 18 out of 19 (94.7%) cases of poorly differentiated prostate adenocarcinoma showed strong nuclear positivity including 9 cases (47.4%) with grade 3 positivity followed by 5 cases (26.3%) with grade 2 positivity and 4 cases (21.0%) were grade 1+ positivity. Only one case (5.3%) was negative with p53.

Table 7: Correlation with both p53 and Ki-67 expression

Prostatic carcinoma	p53 negative and Ki-67 Negative No. (%)	P53 Negative and Ki-67 positive No. (%)	P53 positive and Ki-67 Negative No. (%)	p53 positive and Ki-67 positive No. (%)
Well differentiated adenocarcinoma Low grade (n=5)	3 (60%)	0%	2 (40%)	0%
Moderately differentiated adenocarcinoma Intermediate grade (n=36)	3 (8.3%)	8 (22.2%)	12 (33.4%)	13 (36.1%)
Poorly differentiated adenocarcinoma high grade (n=19)	1 (5.3%)	5 (26.3%)	1 (5.3%)	12 (63.1%)
Total (n=60)	7 (11.6%)	13 (21.7%)	15 (25%)	25 (41.7%)

41.7% cases (25/60) exhibiting positivity for both p53 and Ki-67 marker. Out of total 5 cases of well differentiated adenocarcinoma, 3 cases (60%) were both p53 and Ki-67 negative, 2 cases (40%) were p53 positive and Ki-67 negative. Of total 36 cases of moderately differentiated adenocarcinoma, 3 cases (83%) were both p53 and Ki-67 negative, 8 cases (22.2%) were p53 negative and Ki-67 positive, 12 cases (33.4%) were p53 positive and Ki-67 negative, 13 cases (36.1%) were both p53 and Ki-67 positive. Total 19 cases of poorly differentiated adenocarcinoma, 1 cases (52%) were both p53 and Ki-67 negative, 5 cases (26.4%) were p53 negative and Ki-67 positive, 1 cases (52%) were p53 positive and Ki-67 negative, 12 cases (63.2%) were both p53 negative and Ki-67 negative.

DISCUSSION

In context to the cases selected for study, the age of patients ranged from 39 to 86 years with mean age of 67.3+ 8.9 years. Youngest patient was 39 years old and oldest patient was 86 years old. Out of 100 cases, maximum cases (78%) were 60-79 years age group,

ISSN (O): 2687-5365; ISSN (P): 2753-6556

These findings are comparable with the study of Hirachand,[7] Sadhanti et al,[8] who reported 70.3%, 81.39%, in 60-79 years of age group.

On analyzing the benign group, the age group of patients ranges from 39-81 years. Youngest patient was 39year old and oldest patient was 81 years. Majority of cases were in the age group 61-70 years followed by 71-80 years. This corroborates with findings of Sharma A et al,[9] Shirish C et al.[10] Prostatic adenocarcinoma was seen a decade older than these with benign lesion Among malignant prostatic lesions, the age group of patients ranged from 39 to 82 years. Youngest patient was 39 years old and oldest patient was 82 years. Majority of cases were seen in 71-80 years of age group. Our findings are in well accordance with studies of Shirish B D et all 2014.[10] However, Matpurkar et al found most of the cases in 7th decade.[11]

In our study, among benign group, benign prostatic hyperplasia (BPH) was the most common histological lesion encountered (76.7%). Rekhi et al,[12] found low grade PIN (LGPIN) in 18.6% cases of BPH and 5.8% of cases of adenocarcinoma. Puttuswamy et al found found 8 cases of LGPIN in their study which were associated with BPH. Puttuswamy et al also observed 9 cases of high grade PIN (HGPIN) in their study of which 2 HGPIN foci were seen in BPH and 7 were seen associated with adenocarcinoma.[16]

Among malignant lesions, histologically all the 60 cases were prostatic adenocarcinoma. The commonest pattern seen was acinar followed by arrangement of tumor cells in cords, sheets and cribriform pattern. This is in accordance with study Verma R et al,[13] reported adenocarcinoma in 93% of malignant lesions and metastasis in 7% of cases. Out of 14 cases of adenocarcinoma, 13 cases (92.8%) acinar adenocarcinoma and 1 cases of ductal adenocarcinoma was observed.

Adenocarcinomas are classified by taking into account morphological appearance of glandular cells and the glandular pattern. All the malignant cases were graded using Modified Gleasons scoring system. In present study, a gleason score of 7 was seen in 60% of cases. Gleason score of 8 was observed in 25% of cases. Verma R et al found gleason's score of 6 as the commonest pattern observed in 28% of cases followed by Gleason's score of 7 in 26% cases.[13]

In present study, 2 of 5 (40%) well differentiated adenocarcinoma, 27 of 36 (75%) moderately differentiated adenocarcinoma and 18 of 19 (94.1%) poorly differentiated tumors revealed p53 immunopositivity and a statistically significant correlation was observed between p53 expression and increased gleason grade ($P < 0.001$). Thus it was observed that expression and intensity of p53 increased with the increase in grade, our finding are very well corresponds to study of Verma R et al,[13] this is concordance with many other studies also have demonstrated a positive correlation between p53 immunopositivity and higher Gleason's grade with expression of 21% and 39% respectively. This was also reflected by Sasor et al,[14] and and Kaur H et al,[15] however no statistical significant correlation was found between the two.

The present study revealed (41.7%) cases (25/60) exhibiting positivity for both p53 and Ki-67 markers. Further, Ki-67 score in cancer positive for p53 was greater than that found in cancer negative for p53 and a statistically significant correlation was observed between p53 and Ki-67 expression ($P < 0.05$). Our findings are similar to that observed by Puttuswamy et al,[16] Sarkar et al,[17] observed a significant positive correlation between expression of Ki-67 and p53 protein only in low grade prostatic carcinoma.

CONCLUSION

From the present study, it can be concluded that frequency of expression of both p53, a tumor suppressor protein and Ki-67, a cell proliferation marker is significantly up-regulated in malignant lesion as compaired to benign lesion. Since most cases of prostate cancer are diagnosed microscopically before metastatic spresd and among these, few cases have rapid and life threatning outcome, therefore, it indolecent versus aggressive from prostate cancer can be differentiated from each other, we can help parents remarkably, in the current study, p53 and Ki-67 marker were shown to have a strong relationship with increased Gleason grade, which has an important relationship. With the prognosis of prostate cancer therefore, we propose that these markers can be applied along with other prostate cancer prognostic factors. However, further studies on larger sample are required to elucidate their role in the identification of premalignant lesion.

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ISSN (O): 2687-5365; ISSN (P): 2753-6556