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Original Research Article

COMPARATIVE DIAGNOSTIC UTILITY OF P63 AND HIGH MOLECULAR WEIGHT CYTOKERATIN IN PROSTATIC LESIONS: A PROSPECTIVE OBSERVATIONAL STUDY FROM A NORTH INDIAN TERTIARY CARE CENTER

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ABSTRACT

Background: Prostate cancer is one of the most common malignancies in men, often posing diagnostic challenges due to small foci and benign mimickers like adenosis and atrophy. Prostatic intraepithelial neoplasia (PIN) is a recognized precursor lesion. Immunohistochemical markers targeting basal cells, such as p63 and High Molecular Weight Cytokeratin (HMWCK), assist in accurate diagnosis. **Aims and Objectives:** This study aimed to evaluate and compare the utility of p63 and HMWCK in distinguishing benign prostatic lesions, high-grade PIN (HGPIN), and prostatic carcinoma, and to assess their expression patterns, sensitivity, and specificity. **Materials and Methods:** A hospital based, prospective observational study was conducted on 91 prostatic specimens (TURP specimens, TRUS-guided biopsy specimens, and prostatectomy specimens) over a one-year period from February 2025 to January 2026 at a tertiary care center in North India after obtaining approval from the Institutional Ethical Approval. H&E staining was performed for histopathological diagnosis, followed by immunohistochemical staining for p63 and HMWCK. Staining patterns (continuous, discontinuous, absent) and intensity were recorded and correlated with the final diagnosis. **Results:** The study included 40 malignant (acinar adenocarcinoma), 38 benign (BPH), and 13 premalignant (HGPIN) cases. p63 showed high sensitivity (95.0%) and specificity (86.27%) for detecting malignancy, with an accuracy of 90.11%. HMWCK demonstrated 92.5% sensitivity and 82.35% specificity, with 86.81% accuracy. Benign lesions predominantly showed continuous, moderate-to-strong staining for both markers. Malignant lesions consistently showed loss of basal cell staining. HGPIN exhibited variable, often discontinuous or patchy staining patterns. A statistically significant association was found between marker expression and lesion categories ($p<0.0001$). **Conclusion:** Both p63 and HMWCK are highly effective immunohistochemical markers for differentiating benign, premalignant, and malignant prostatic lesions. p63 demonstrated slightly superior diagnostic accuracy. Their use, particularly in challenging cases with small foci or equivocal morphology, significantly enhances diagnostic precision.

INTRODUCTION

The prostate gland is increasingly affected by a spectrum of disorders with advancing age, ranging from benign prostatic hyperplasia (BPH) to prostatic intraepithelial neoplasia (PIN) and carcinoma. This continuum has gained clinical importance due to rising life expectancy and the growing elderly male population worldwide. Epidemiological data indicate a marked rise in prostatic diseases, with global BPH cases exceeding 79 million in 2019 and prostate cancer incidence increasing significantly over recent decades. Although BPH is non-malignant, it significantly impacts quality of life through lower urinary tract symptoms, bladder dysfunction, and potential renal complications. Concurrently, prostate cancer represents a major health burden, emphasizing the need for early detection and accurate diagnosis.[1,2]

PIN occupies an intermediate position as a premalignant lesion, reflecting early epithelial changes that may progress to carcinoma.

Recognition of such precursor lesions highlights the importance of evaluating subtle histological alterations rather than relying solely on a benign-versus-malignant framework. Clinically, distinguishing between benign and malignant prostatic conditions is essential, as both may present with similar symptoms such as urinary obstruction. Early diagnosis of malignancy improves treatment outcomes, while timely identification of benign conditions helps prevent complications and unnecessary interventions. Increasing disease burden also challenges healthcare systems in terms of screening, diagnostic pathways, and resource allocation.[3]

Diagnosis of prostatic lesions often relies on needle biopsies, which may show small glandular proliferations that closely mimic carcinoma. Morphological assessment alone can be insufficient, especially in limited or equivocal samples. A key histological feature in differentiating benign from malignant glands is the presence or absence of the basal cell layer. Benign glands retain a continuous basal cell layer, whereas invasive carcinoma is characterized by its absence. However, this distinction may not always be clearly visible on routine hematoxylin and eosin staining, necessitating the use of immunohistochemistry (IHC) for confirmation.[4,5]

Atypical small acinar proliferation (ASAP) represents a diagnostic challenge, occurring in approximately 4–5% of prostate biopsies. It describes lesions suspicious for carcinoma but lacking definitive features. Studies show that 25–50% of patients with ASAP are later diagnosed with prostate cancer on repeat biopsy, highlighting its clinical significance. Differentiating ASAP from benign mimickers such as atrophy or adenosis is difficult, particularly when basal cell staining is inconclusive. Therefore, careful follow-up and repeat evaluation are often recommended.[6,7]

The basal cell layer plays a central role in prostate pathology. In normal glands, it forms a continuous layer beneath luminal cells, serving as a hallmark of benignity. Its absence is a defining feature of invasive carcinoma. Immunohistochemical markers such as p63 and high molecular weight cytokeratin (HMWCK) are widely used to demonstrate basal cells. These markers help confirm benign lesions when positive and support malignancy when absent, although interpretation must consider potential pitfalls such as patchy staining in benign conditions.[8,9]

The evolution of IHC has significantly enhanced diagnostic accuracy in prostate pathology. Initially dependent on morphology, pathology practice now incorporates basal cell markers and tumour-associated markers such as AMACR and NKX3.1. Modern approaches often use multiparametric panels to improve reliability, particularly in small or ambiguous lesions. This progression reflects the increasing complexity of prostate diagnostics and the need for objective, reproducible methods.[10,11]

Among basal cell markers, p63 is a nuclear transcription factor specifically expressed in basal cells. It shows strong and consistent staining in benign glands and is typically absent in carcinoma, making it highly sensitive and specific. Studies report high diagnostic accuracy, with p63 demonstrating fewer false positives compared to other markers. Nevertheless, rare exceptions and patchy staining in benign conditions necessitate cautious interpretation.[12]

HMWCK, detected using the 34βE12 antibody, stains the cytoplasm of basal cells and is another important marker. It highlights the basal layer in benign glands and is absent in most carcinomas. While its cytoplasmic staining provides useful morphological context, interpretation can be challenging in small or poorly oriented biopsy samples. Occasional staining variability and rare positivity in malignant glands further emphasize the need to correlate findings with morphology and additional markers. Despite these limitations, HMWCK remains a valuable adjunct in distinguishing benign from malignant prostatic lesions.[13]

This study aimed to evaluate the diagnostic utility of p63 and high molecular weight cytokeratin (HMWCK) immunohistochemistry in differentiating benign prostatic lesions, precursor lesions, and prostatic carcinoma. It sought to accurately identify benign, precursor, and malignant lesions while assessing the specific roles of p63 and HMWCK in distinguishing prostatic intraepithelial neoplasia from other entities. The objectives included analysing their expression patterns and determining the sensitivity and specificity of both markers in reliably diagnosing various prostatic pathologies.

MATERIALS AND METHODS

This hospital-based prospective observational study was carried out at GSVM Medical College and LLR & Associated Hospitals, Kanpur, a major tertiary care center, over a one-year period from February 2025 to January 2026 after obtaining approval from the Ethics Committee (For Biomedical Heath & Research), G.S.V.M. Medical College, Kanpur, India (IEC Ref No. EC/BMHR/2024/203).

Study Population

The study population comprised 91 patients with clinically suspected prostatic disease who underwent diagnostic or therapeutic procedures at the Department of Surgery during the study period.

Inclusion Criteria

All cases of radical prostatectomy specimens, TURP specimens, and TRUS-guided needle core biopsies were included in the study.

Exclusion Criteria

Cases with tissue loss during retrieval, insufficient biopsy material, or patients who had undergone prior chemotherapy or radiotherapy were excluded from the study.

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Data Analysis

Data were systematically collected using a structured proforma for 91 cases, recording age, specimen type, histopathological diagnosis, basal cell visibility on H&E, and p63 and HMWCK immunohistochemistry findings, including staining result, pattern, and intensity. All data were entered into Microsoft Excel and verified for accuracy. Statistical analysis was performed using SPSS, applying descriptive statistics and Chi-square tests, with results presented in tables and graphs, and p-value <0.05 considered statistically significant.

RESULTS

In this study of 91 cases, the mean age was 70.70 ± 9.01 years (range 55–85), with a median of 71 years and an interquartile range of 14.5, indicating moderate variability and an approximately symmetric, near-normal age distribution predominantly within 60–80 years. Most specimens were obtained via TURP (61.54%), followed by TRUS biopsies (34.07%) and prostatectomy (4.40%). Malignant lesions (43.96%) slightly outnumbered benign cases (41.76%), while premalignant HGPIN accounted for 14.29%, reflecting a balanced spectrum and highlighting the importance of accurate diagnostic differentiation.

Table 1: Association Between Basal Cell Identification on H&E and Immunohistochemical Staining Patterns (P63 and HMWCK)

Basal cells on H&E	p63 Staining Pattern			Chi-square	p-value
	Absent	Continuous	Discontinuous		
Identifiable	6	25	12	53.0058	<0.0001
Not seen	24	0	1		
Doubtful	16	1	6		

There is a highly significant association between basal cell identification on H&E staining and P63 immunohistochemical patterns ($\chi^2 = 53.0058$, $p < 0.0001$), with identifiable basal cells mostly showing continuous staining, while non-identifiable cases largely show absent staining. Doubtful cases exhibit mixed patterns but tend toward absent staining, highlighting reduced reliability of basal cell detection on H&E alone compared to immunohistochemistry.

There is a highly significant association between basal cell identification on H&E and HMWCK staining patterns ($\chi^2 = 46.5282$, $p < 0.0001$), with identifiable basal cells mainly showing continuous staining, while non-identifiable cases predominantly show absent staining. Doubtful cases largely exhibit absent staining with occasional patchy or minimal continuous patterns, indicating limited reliability of H&E alone compared to immunohistochemistry.

Table 2: Frequency and percentage distribution of different glandular architecture patterns observed in the study samples

Glandular Architecture	Count (n)	Percent (%)
Predominantly Discrete smaller glands	17	42.5
Fused, cribriform and irregular glands	12	30.0
Predominantly Sheets and cords of tumor cells	6	15.0
Predominantly fused and irregular glands	5	12.5

Predominantly discrete small glands were the most common glandular architecture pattern among malignant cases (42.5%), followed by fused, cribriform, and irregular glands (30%). Less frequent patterns included sheets and cords of tumor cells (15%) and predominantly fused irregular glands (12.5%), indicating variability in tumor morphology.

Table 3: Association of p63 immunohistochemical expression with benign, premalignant, and malignant prostatic lesions

Lesion Category	P63 result		Chi-Square	p-value
	Negative	Positive		
Benign	2	36	63.5100	<0.0001
Malignant	38	2		
Premalignant	5	8		

There is a highly significant association between p63 expression and lesion category ($\chi^2 = 63.51$, $p < 0.0001$), with benign lesions predominantly showing p63 positivity and malignant lesions largely showing loss of expression. Premalignant lesions display mixed results with partial retention of p63, often in a discontinuous or patchy pattern, reflecting intermediate basal cell preservation.

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Table 4: Association of High Molecular Weight Cytokeratin (HMWCK) expression with benign, premalignant, and malignant prostatic lesions

Lesion Category	HMWCK result		Chi-Square	p-value
	Negative	Positive		
Benign	1	37	63.6942	<0.0001
Malignant	37	3		
Premalignant	8	5		

There is a highly significant association between HMWCK expression and lesion category (χ^2 = 63.6942, $p < 0.0001$), with benign lesions mostly showing positivity and malignant lesions predominantly showing loss of expression. Premalignant lesions exhibit variable HMWCK expression, indicating inconsistent basal cell layer preservation between benign and malignant states.

Table 5: Distribution of p63 staining intensity across benign, premalignant, and malignant prostatic lesions

Lesion Category	p63 Intensity				Chi-Square	p-value
	Absent	Moderate	Strong	Weak		
Benign	2	19	17	0	87.2841	<0.0001
Malignant	38	0	0	2		
Premalignant	5	3	1	4		

There is a highly significant association between p63 staining intensity and lesion category (χ^2 = 87.2841, $p < 0.0001$), with benign lesions showing predominantly moderate to strong intensity and malignant lesions largely showing absent staining. Premalignant lesions display variable intensity patterns, indicating partial and inconsistent basal cell preservation between benign and malignant conditions.

Table 6: Distribution of p63 staining patterns across benign, premalignant, and malignant prostatic lesions

Lesion Category	P63 Staining Pattern			Chi-Square	p-value
	Absent	Continuous	Discontinuous		
Benign	3	26	9	81.2183	<0.0001
Malignant	38	0	2		
Premalignant	5	0	8		

There is a highly significant association between p63 staining pattern and lesion category (χ^2 = 81.2183, $p < 0.0001$), with benign lesions predominantly showing continuous staining and malignant lesions showing absence of staining. Premalignant lesions demonstrate mainly discontinuous or absent patterns, indicating partial basal cell loss and aiding differentiation between benign and malignant lesions.

There is a highly significant association between HMWCK staining intensity and lesion category (χ^2 = 82.33, $p < 0.0001$), with benign lesions showing predominantly moderate to strong intensity and malignant lesions largely showing absent staining. Premalignant lesions exhibit variable intensity patterns, indicating partial basal cell preservation and supporting differentiation between benign and malignant lesions.

The study demonstrated a highly significant association between HMWCK staining pattern and lesion category (χ^2 = 78.49, $p < 0.0001$), indicating its strong diagnostic relevance. Continuous staining was predominantly seen in benign lesions, reflecting intact basal cell layers, whereas malignant lesions largely showed absence of staining, consistent with basal cell loss, with only occasional patchy positivity. Premalignant lesions exhibited mainly absent or patchy staining, suggesting partial basal cell disruption. Additionally, marker positivity analysis revealed that most benign cases were positive for both p63 (36 cases) and HMWCK (37 cases), while malignant cases showed minimal positivity (p63: 2, HMWCK: 3). Premalignant lesions demonstrated intermediate expression, with p63 positivity in 8 cases and HMWCK in 5 cases, reflecting their transitional biological nature.

Table 7: Distribution of negative immunohistochemical staining results for p63 and HMWCK across benign, premalignant, and malignant prostatic lesions

Lesion Category	Negative	
	P63 result	HMWCK result
Benign	2	1
Malignant	38	37
Premalignant	5	8

Immunohistochemical negativity for p63 and HMWCK is strongly associated with malignant lesions, with most malignant cases showing loss of both markers, while benign lesions rarely exhibit negativity. Premalignant lesions show intermediate negativity, reflecting partial basal cell loss and supporting the diagnostic value of these markers in distinguishing lesion categories.

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Table 8: Sensitivity, specificity, predictive values, and overall accuracy of p63 and HMWCK in diagnosing prostatic carcinoma

Marker	Sensitivity(%)	Specificity(%)	PPV(%)	NPV(%)	Accuracy(%)
P63	95.0	86.27	84.44	95.65	90.11
HMWCK	92.5	82.35	80.43	93.33	86.81

p63 showed slightly superior diagnostic performance compared to HMWCK in detecting prostatic carcinoma, with higher sensitivity (95.0%), specificity (86.27%), and overall accuracy (90.11%), while HMWCK also demonstrated strong but slightly lower values. Both markers are highly reliable, where loss of basal cell staining strongly indicates malignancy, and their combined use enhances diagnostic confidence in differentiating malignant from non-malignant prostatic lesions.

DISCUSSION

Prostatic lesions present a considerable diagnostic challenge in routine surgical pathology, particularly in needle biopsy specimens where small foci of crowded glands, mild cytological atypia, and architectural overlap between benign mimickers and early carcinoma are frequently encountered.[8] Benign conditions such as adenosis, basal cell hyperplasia, and atrophic changes can closely resemble carcinoma, while high-grade prostatic intraepithelial neoplasia (HGPIN) represents a recognized precursor lesion positioned between benign and malignant states. HGPIN is considered the most likely precursor of prostatic adenocarcinoma and requires accurate identification for appropriate clinical management.[14]

A key distinguishing feature between benign and malignant prostatic glands is the presence or absence of the basal cell layer. Benign acini retain an intact basal layer, whereas invasive carcinoma is characterized by its absence. However, this distinction may not always be clearly evident on routine hematoxylin and eosin staining, particularly in limited or fragmented biopsy material. In such cases, immunohistochemistry plays a crucial role by highlighting basal cells using markers such as p63, which stains basal cell nuclei, and high molecular weight cytokeratin (HMWCK), which stains their cytoplasm.[15]

Among these markers, p63 offers practical advantages due to its distinct nuclear staining pattern, which allows easier interpretation in small or crowded glands. Previous studies have also demonstrated that p63 has higher sensitivity in detecting basal cells compared to HMWCK, particularly in challenging specimens such as transurethral resection tissues, where it shows superior staining consistency. HMWCK, although well established and valuable, may show patchy staining, background artifacts, or occasional false negatives in benign glands. Rarely, focal positivity may even be observed in carcinoma, highlighting the need for cautious interpretation and correlation with morphology.[16]

The diagnostic complexity is further increased by precursor and intraductal lesions. HGPIN typically retains basal cells and therefore shows positivity for both p63 and HMWCK, unlike invasive carcinoma, which lacks basal cell staining. However, variability in staining patterns may occur, and some lesions may show discontinuous or patchy expression. Therefore, current diagnostic practice favors a panel approach combining basal cell markers with tumor-associated markers such as AMACR to improve diagnostic accuracy.[17]

In this context, comparative evaluation of p63 and HMWCK is important, as both markers address the same diagnostic principle but differ in sensitivity, staining characteristics, and interpretive clarity. Their combined use improves diagnostic confidence, especially in limited biopsy material, reducing the risk of both overdiagnosis and underdiagnosis.[15] While the general diagnostic utility of p63 and HMWCK is well-established globally, their comparative performance requires validation within specific regional demographics. There remains a paucity of prospective data evaluating these markers in the North Indian population, particularly within high-volume, resource-constrained tertiary care settings. Consequently, this study provides a pragmatic assessment of how these markers perform on routinely encountered, often fragmented TURP and TRUS biopsy specimens. Establishing the most robust marker under these real-world conditions is essential for guiding cost-effective, single-marker institutional protocols that maintain high diagnostic accuracy.

The study demonstrated that prostatic lesions predominantly affect elderly men, with a mean age of approximately 70 years, reflecting their higher prevalence in older populations. The majority of specimens were obtained through TURP and needle biopsies, which are often limited and fragmented, making accurate diagnosis challenging. Previous studies have similarly shown that both p63 and HMWCK are consistently expressed in benign lesions and absent in malignant lesions, with p63 demonstrating slightly higher sensitivity.[13,18]

The lesion distribution in the study included benign, premalignant, and malignant categories, providing a representative spectrum for evaluating basal cell markers. Consistent with previous research, both p63 and HMWCK were expressed in benign and premalignant lesions, while malignant lesions lacked expression. These findings reinforced the importance of basal cell markers in distinguishing carcinoma from its mimickers and confirm their statistical significance in diagnostic practice.[13,17]

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A strong correlation was observed between basal cell identification on routine histology and immunohistochemical staining patterns. Continuous staining was associated with benign lesions, while absent staining was characteristic of malignancy. Multiple studies confirm the reliability of these markers in reflecting true basal cell status.[13,17]

Morphologically, malignant lesions showed a range of architectural patterns, including small discrete glands, fused glands, and cribriform structures. These features are often associated with loss of the basal cell layer, which was confirmed by negative staining for both markers. Similar studies have demonstrated that absence of basal cell staining is a strong indicator of malignancy and can alter diagnosis in suspicious cases.[13,19]

Both p63 and HMWCK showed highly significant associations with lesion categories, with benign lesions demonstrating strong positivity and malignant lesions showing predominant negativity. Premalignant lesions exhibited variable expression, reflecting partial basal cell disruption. These findings are consistent with earlier studies that highlight the diagnostic utility of basal cell markers in distinguishing benign, premalignant, and malignant lesions.[13,17,20]

Staining intensity and pattern further supported these observations, with benign lesions showing continuous, moderate-to-strong staining, premalignant lesions showing patchy or discontinuous staining, and malignant lesions showing absence of staining. This progressive loss of basal cell integrity corresponds to the biological progression from benign to malignant states and has been validated in previous studies.[21-23]

Overall, both p63 and HMWCK proved to be reliable markers for identifying basal cell presence and distinguishing prostatic lesions. However, p63 demonstrated slightly superior diagnostic performance, with higher sensitivity, specificity, and overall accuracy compared to HMWCK. This may be attributed to its nuclear staining pattern, which is easier to interpret and less affected by technical artifacts.[13,24]

While both markers are highly effective in prostate pathology, p63 offers a marginal advantage in difficult diagnostic scenarios. Their combined use, along with careful morphological assessment, significantly enhances diagnostic accuracy and ensures appropriate classification of prostatic lesions in routine practice.[24]

Limitations

While this study provides valuable prospective data on the utility of basal cell markers in a resource-conscious setting, it is not without limitations. First, as a single-center study with a cohort of 91 cases over a one-year period, the findings reflect a specific regional demographic and may not be universally generalizable. Second, the evaluation was restricted to histopathological and immunohistochemical parameters. The study did not incorporate molecular or genomic profiling, nor did it include long-term clinical follow-up to track disease progression, particularly within the HGPIN cohort. Future large-scale, multi-center studies integrating molecular diagnostics and longitudinal tracking are warranted to further validate these findings.

CONCLUSION

Based on analysis of 91 prostatic specimens, p63 and HMWCK are highly effective immunohistochemical markers correlating with basal cell integrity across lesions. Benign hyperplasia shows continuous staining, carcinoma shows absence, and HGPIN displays patchy patterns. p63 demonstrates slightly higher sensitivity, specificity, and accuracy than HMWCK, though both have high negative predictive value (>93%) for excluding malignancy. They are complementary tools, and combined use with morphological correlation significantly improves diagnostic accuracy in differentiating benign, premalignant, and malignant prostatic lesions, aiding appropriate patient management.

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Ethical Approval & Consent to Participate

All necessary consent & approval was obtained by authors.

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