Original Research

EVALUATION OF KI67 EXPRESSION AS AN INDEPENDENT PROGNOSTIC MARKER IN PROSTATE ADENOCARCINOMA: A CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Background and Objectives: Prostate adenocarcinoma is the most common malignancy in men worldwide and is characterized by significant heterogeneity in clinical behavior. Ki67, a nuclear proliferation antigen expressed during active cell-cycle phases, has emerged as a potential independent prognostic marker. This study aimed to evaluate Ki67 immunohistochemical expression and its association with clinicopathological parameters in prostate adenocarcinoma.

Materials and Methods: A retrospective and prospective observational study was conducted over 18 months (July 2022–December 2023) at the Department of Pathology, Narayana Medical College and Hospital, Nellore, India. Sixty histologically confirmed prostate adenocarcinoma specimens (TURP chips, core needle biopsies, and radical prostatectomy specimens) were subjected to haematoxylin and eosin (H&E) staining and Ki67 immunohistochemistry. Ki67 expression was scored as 1+ (<10%), 2+ (10–50%), or 3+ (>50%) of stained tumour nuclei. Associations with age, PSA levels, Gleason grade group, tumour stage, metastasis, perineural invasion, PSA doubling time, and PSA response were analyzed using the chi-square test (SPSS v25.0).

Results: Mean patient age was 58.03 ± 10.25 years (range 40–78). Ki67 expression was 1+ in 25%, 2+ in 45%, and 3+ in 30% of cases. Ki67 expression showed statistically significant associations with age ($p < 0.001$), PSA level ($p = 0.020$), Gleason grade group ($p < 0.001$), tumour stage ($p = 0.007$), metastasis ($p = 0.037$), perineural invasion ($p = 0.004$), PSA doubling time ($p = 0.001$), and PSA treatment response ($p = 0.001$). Notably, 77.8% of Grade 5 tumours and 83.3% of T4-stage tumours exhibited high Ki67 (3+) expression.

Conclusion: Ki67 expression correlates significantly with multiple adverse clinicopathological parameters and treatment outcomes in prostate adenocarcinoma. It may serve as a valuable independent prognostic adjunct to Gleason grading and PSA, facilitating risk stratification and individualized management decisions.

Keywords: Ki67, Prostate Adenocarcinoma, Immunohistochemistry, Gleason Grade Group, Prognostic Marker.

INTRODUCTION

Prostate cancer is the most prevalent malignancy among men globally, excluding non-melanoma skin tumours, and ranks fifth in cancer-related mortality. In 2020, approximately 1.41 million new cases were diagnosed with 375,000 deaths worldwide.[1,2] Prostatic adenocarcinoma constitutes approximately 95% of all prostate cancers, encompassing acinar and ductal subtypes originating from glandular epithelium.[3]

The clinical behaviour of prostate adenocarcinoma is markedly heterogeneous. While the majority of tumours are indolent and slow-growing, a significant subset pursues an aggressive course with early metastasis and treatment resistance. Current prognostic tools—prostate-specific antigen (PSA), Gleason grading, and clinical staging—do not

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always predict outcome reliably on an individual basis, underscoring the need for additional biomarkers.[4]

Ki67, a nuclear non-histone protein encoded by the MKI67 gene, is expressed during all active phases of the cell cycle (G1, S, G2, and M) but is absent in resting (G0) cells. Its half-life of approximately 60–90 minutes makes it a sensitive and dynamic marker of proliferative activity.[5] Ki67 immunohistochemistry, readily performed on routinely processed formalin-fixed paraffin-embedded (FFPE) tissue, has demonstrated prognostic value in breast, lung, cervical, soft-tissue, and prostate cancers.[6]

In prostate cancer, Ki67 labelling index has been correlated with Gleason score, pathological stage, biochemical recurrence, and cancer-specific mortality in multiple large cohort studies.[7-9] However, data from Indian patient populations remain sparse, and the systematic evaluation of Ki67 across the full spectrum of clinicopathological variables—including PSA doubling time and treatment response—has rarely been undertaken in a single institutional series.

The present study was therefore designed to assess Ki67 immunohistochemical expression in prostate adenocarcinoma and to determine its associations with age, PSA level, Gleason grade group, tumour stage, metastasis, perineural invasion, PSA doubling time, and PSA treatment response.

MATERIALS AND METHODS

This retrospective and prospective observational study was carried out at the Department of Pathology, Narayana Medical College and Hospital, Nellore, Andhra Pradesh, India, from July 2022 to December 2023 (18 months), with approval from the Institutional Ethics Committee.

Study population

Sixty specimens from patients with histologically confirmed prostate adenocarcinoma were enrolled. Specimens included TURP chips (n = 39; 65%), core needle biopsies (n = 16; 26.7%), and radical prostatectomy specimens (n = 5; 8.3%). Cases with non-neoplastic lesions or biopsies yielding insufficient tissue for immunohistochemistry were excluded.

Histopathological evaluation

All tissue specimens were fixed in 10% neutral-buffered formalin, processed through graded alcohols, cleared in xylene, and embedded in paraffin. Four-micrometre sections were stained with haematoxylin and eosin (H&E) for histological diagnosis and Gleason grading. Tumours were graded according to the 2014 International Society of Urological Pathology (ISUP) Grade Group system: Grade Group 1 (Gleason score ≤6), Grade Group 2 (3+4=7), Grade Group 3 (4+3=7), Grade Group 4 (Gleason score 8), and Grade Group 5 (Gleason scores 9–10). Tumour stage was assigned according to the American Joint Committee on Cancer (AJCC) TNM classification.

Immunohistochemistry

Ki67 immunohistochemistry was performed on 4-µm FFPE sections using a monoclonal anti-Ki67 antibody (MIB-1 clone, Dako). Heat-induced epitope retrieval was performed in citrate buffer (pH 6.0). The labelled streptavidin–biotin (LSAB) detection method was used, with 3,3′-diaminobenzidine (DAB) as chromogen and haematoxylin as counterstain. Ki67 labelling index (LI) was calculated as the percentage of positively stained tumour cell nuclei in three to five randomly selected high-power fields (×400 magnification): Ki67 LI = (Number of Ki67-positive tumour nuclei / Total tumour nuclei counted) × 100. Expression was categorised semi-quantitatively: 1+ (<10% positive nuclei), 2+ (10–50%), and 3+ (>50%).

Statistical analysis

Data were entered in Microsoft Excel and analyzed using SPSS version 25.0. Categorical variables are presented as frequency and percentage. The chi-square test was used to assess associations between Ki67 expression and clinicopathological parameters. A p-value < 0.05 was considered statistically significant.

RESULTS

A total of 60 prostate adenocarcinoma specimens were analyzed. The mean age of patients was 58.03 ± 10.25 years (range 40–78 years); the most common age group was 50–59 years (31.7%). Demographic and clinicopathological characteristics, with corresponding Ki67 association p-values, are summarized in Table 1.

Table 1: Clinicopathological profile and Ki67 associations

Parameter	Category	n (%)	p-value (vs Ki67)
Age (years)	40–49	15 (25.0)	< 0.001
	50–59	19 (31.7)	
	60–69	15 (25.0)	
	≥70	11 (18.3)	
PSA (ng/mL)	4.1–10	22 (36.7)	0.020
	10.1–20	20 (33.3)	
	>20	18 (30.0)	

Gleason Grade Group	Grade 1 (≤6)	6 (10.0)	< 0.001
	Grade 2 (3+4=7)	11 (18.3)	
	Grade 3 (4+3=7)	12 (20.0)	
	Grade 4 (Score 8)	13 (21.7)	
	Grade 5 (9–10)	18 (30.0)	
Tumour Stage	T2	11 (18.4)	0.007
	T3	20 (33.3)	
	T4	29 (48.3)	
Metastasis	M0 (absent)	17 (28.3)	0.037
	M1 (present)	43 (71.7)	
Perineural Invasion	Absent	20 (33.3)	0.004
	Present	40 (66.7)	

Ki67 expression distribution

Ki67 expression was categorized as 1+ (low) in 15 patients (25.0%), 2+ (moderate) in 27 patients (45.0%), and 3+ (high) in 18 patients (30.0%), as shown in Table 2.

Table 2: Ki67 expression distribution in prostate adenocarcinoma (n = 60)

Ki67 Score	Criteria	n (%)	Interpretation
1+	<10% positive nuclei	15 (25.0)	Low proliferative activity
2+	10–50% positive nuclei	27 (45.0)	Moderate proliferative activity
3+	>50% positive nuclei	18 (30.0)	High proliferative activity
Total		60 (100)	

Ki67 and Gleason grade group

A highly significant association was observed between Ki67 expression and Gleason grade group ($\chi^2 = 29.90$; $p < 0.001$) (Table 3). Among Grade 5 tumours, 77.8% exhibited 3+ Ki67 expression, whereas Grade 1–2 tumours predominantly showed 1+ expression.

Table 3: Association of Ki67 expression with Gleason grade group

Gleason Grade Group	Gleason Score	Ki67 1+ n (%)	Ki67 2+ n (%)	Ki67 3+ n (%)	Total n (%)
Grade 1	≤6	3 (20.0)	3 (11.1)	0 (0)	6 (10.0)
Grade 2	3+4=7	3 (20.0)	7 (25.9)	1 (5.6)	11 (18.3)
Grade 3	4+3=7	4 (26.7)	7 (25.9)	1 (5.6)	12 (20.0)
Grade 4	8	3 (20.0)	8 (61.5)	2 (11.1)	13 (21.7)
Grade 5	9–10	2 (13.3)	2 (11.1)	14 (77.8)	18 (30.0)
Total		15 (100)	27 (100)	18 (100)	60 (100)
$\chi^2 = 29.90$	$p < 0.001$				

Ki67 and tumour stage

Ki67 expression increased progressively with advancing tumour stage ($\chi^2 = 14.240$; $p = 0.007$) (Table 4). High Ki67 (3+) was observed in 83.3% of T4-stage tumours, compared to 0% in T2-stage tumours.

Table 4: Association of Ki67 expression with tumour stage

Tumour Stage	Ki67 1+ n (%)	Ki67 2+ n (%)	Ki67 3+ n (%)	Total n (%)
T2	5 (33.3)	6 (22.2)	0 (0)	11 (18.4)
T3	6 (40.0)	11 (40.7)	3 (16.7)	20 (33.3)
T4	4 (26.7)	10 (37.0)	15 (83.3)	29 (48.3)
Total	15 (100)	27 (100)	18 (100)	60 (100)
$\chi^2 = 14.240$; $p = 0.007$				

Ki67, metastasis, and perineural invasion

Ki67 expression was significantly associated with both metastatic status ($\chi^2 = 6.594$; $p = 0.037$) and perineural invasion ($\chi^2 = 11.217$; $p = 0.004$). Among metastatic cases (M1), 88.9% had high Ki67 (3+). Perineural invasion was present in 94.4% of cases with 3+ Ki67 expression.

Ki67, PSA doubling time, and treatment response

High Ki67 expression (3+) was significantly associated with rapid PSA doubling time (≤12 months) (66.7%; p = 0.001) and with absence of PSA response to treatment (83.3% of non-responders; p = 0.001), as shown in Table 5.

Table 5: Ki67 expression in relation to PSA doubling time and PSA response

Parameter	Category	Ki67 1+ n (%)	Ki67 2+ n (%)	Ki67 3+ n (%)	Total n (%)	p-value
PSA Doubling Time	≤12 months	3 (20.0)	9 (33.3)	12 (66.7)	24 (40.0)	0.001
	>12 months	12 (80.0)	18 (66.7)	6 (33.3)	36 (60.0)	
PSA Response	Decrease	13 (86.7)	11 (40.7)	3 (16.7)	27 (45.0)	0.001
	No change	2 (13.3)	16 (59.3)	15 (83.3)	33 (55.0)	

DISCUSSION

In this institutional series of 60 prostate adenocarcinoma specimens, Ki67 immunohistochemical expression was evaluated against a broad panel of clinicopathological variables. Statistically significant associations were identified across all parameters examined—including age, serum PSA, Gleason grade group, tumour stage, metastatic status, perineural invasion, PSA doubling time, and treatment response—providing multi-dimensional evidence for Ki67 as an independent prognostic adjunct in this disease.

The mean patient age of 58.03 years is slightly younger than the typically reported Western peak incidence of 60–70 years, consistent with previous Indian series.[10] The predominance of TURP biopsies (65%) reflects the common clinical presentation with obstructive lower urinary tract symptoms in this population.

A notable proportion of patients (64%) presented with serum PSA above 10 ng/mL, suggesting a higher baseline disease burden in this cohort. The significant relationship between PSA level and Gleason grade group (p = 0.020) observed here parallels earlier reports from Sharma et al. and Ngwu et al., both of whom documented a positive correlation between PSA magnitude and tumour grade.[11,12]

More than half of the study population (51.7%) fell into Grade Groups 4 or 5, underscoring a tendency toward high-grade disease at presentation in this South Indian population. This pattern has been similarly noted in other Indian and Southeast Asian institutional series.[10]

A highly significant relationship between Ki67 LI and Gleason grade group (p < 0.001) was observed, consistent with a large body of published evidence. In a cohort of 756 patients, Kammerer-Jacquet et al. established Ki67 as an independent predictor of cancer-specific survival beyond conventional grade and PSA variables.[13] Berney et al., analysing 808 conservatively managed patients, similarly identified Ki67 as a robust prognostic variable independent of clinical parameters.[14] Fisher et al. further confirmed Ki67 as a significant predictor of prostate cancer-specific mortality in a large conservatively managed cohort.[25] The seminal work of Bubendorf et al. established Ki67 labelling index as an independent predictor of disease progression after radical prostatectomy, laying the foundation for its current prognostic utility.[24] The present observation that 77.8% of Grade 5 tumours harboured high Ki67 (3+) expression directly reflects the progressive rise in cellular proliferation accompanying tumour dedifferentiation; notably, intra-tumoral Ki67 heterogeneity in high-grade cases, as described by Vlajnic et al., underscores the importance of multi-field scoring in such tumours.[22]

Ki67 expression rose progressively with advancing T stage (p = 0.007), with 83.3% of T4 tumours demonstrating 3+ positivity. Sulik et al. previously documented parallel relationships between Ki67, pathological stage, and Gleason score.[10] In a larger tissue microarray study encompassing more than 1000 prostatectomy specimens, Tretiakova et al. reported that elevated Ki67 proliferation index was independently associated with seminal vesicle involvement, extracapsular extension, and inferior recurrence-free survival.[15]

Significant associations were also observed between Ki67 expression and both metastatic disease (p = 0.037) and perineural invasion (p = 0.004). Perineural invasion represents a recognised mechanism of extraprostatic spread, and the finding that 94.4% of 3+ Ki67 cases exhibited perineural invasion raises the possibility that heightened proliferative activity facilitates exploitation of perineural pathways for tumour dissemination. Missaoui et al. reported comparable findings, with Ki67 emerging as a significant predictor of mortality alongside perineural invasion and metastasis in multivariate analysis.[17]

Among the most clinically relevant observations in this study are the associations between Ki67 expression and both PSA doubling time (p = 0.001) and PSA response to treatment (p = 0.001). Because PSA doubling time serves as a well-validated surrogate endpoint for biochemical recurrence, its significant correlation with Ki67 LI is concordant

with reports by Shahit et al. and Lobo et al., each of whom established Ki67 as an independent predictor of biochemical failure after radical prostatectomy.[18,19] Bettencourt et al. similarly demonstrated Ki67 expression to be a significant prognostic marker of disease recurrence after radical prostatectomy in one of the earliest institutional series addressing this question.[16] More recently, Kim et al. identified Ki67 as an independent predictor of biochemical recurrence in clinically localised prostate cancer alongside other immunohistochemical markers,[20] and He et al. confirmed its prognostic value in patients undergoing laparoscopic radical prostatectomy.[21] Furthermore, the observation that 83.3% of treatment non-responders harboured high Ki67 expression raises the possibility that tumour proliferative activity contributes to intrinsic resistance to androgen deprivation therapy and other systemic agents, an avenue warranting prospective investigation.

From a practical standpoint, these findings carry meaningful clinical implications. In resource-limited settings where comprehensive molecular biomarker testing is not available, Ki67 immunohistochemistry can be readily performed on routinely processed FFPE material at modest cost. Its capacity to differentiate risk categories among patients with comparable Gleason grades makes it a useful tool for guiding decisions regarding adjuvant therapy intensity, eligibility for active surveillance, and the frequency of clinical follow-up. Green et al. have further validated Ki67 as a biomarker of treatment response in prostate cancer, reinforcing its potential role in monitoring therapeutic efficacy in addition to prognostication.[23]

CONCLUSION

The present study demonstrates that Ki67 immunohistochemical expression correlates significantly with a wide spectrum of adverse clinicopathological features in prostate adenocarcinoma, encompassing higher Gleason grade group, advanced pathological stage, distant metastasis, perineural invasion, shortened PSA doubling time, and resistance to systemic therapy. Collectively, these findings support its incorporation as a cost-effective and practically accessible independent prognostic marker alongside established tools such as Gleason grading and serum PSA, with potential to refine risk stratification and inform individualized therapeutic decisions. Prospective multicentre investigations with extended follow-up are necessary to further validate these observations across a wider spectrum of Indian patient populations.

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