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Original Article

# Ki-67 Proliferative Index and Its Association with ISUP Grade Group in Prostatic Adenocarcinoma: A Cross-Sectional Immunohistochemical Study

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## ABSTRACT

**Background**: Histological grade remains central to prostate cancer risk stratification, but tumors within the same grade group may differ biologically. Ki-67 immunohistochemistry provides a practical estimate of proliferative activity and may complement routine morphology.
**Objective**: To determine Ki-67 expression in prostatic adenocarcinoma and evaluate its association with the International Society of Urological Pathology (ISUP) grade group.
**Methods**: This hospital-based cross-sectional study included 50 histologically confirmed cases of prostatic adenocarcinoma diagnosed in the Department of Pathology at a tertiary care centre. Hematoxylin-and-eosin sections were graded using the modified Gleason/ISUP system. Ki-67 nuclear staining was assessed in tumor cells and categorized as low (<10%) or high (≥10%). Associations were evaluated using the chi-square test; an ordinal trend was examined using Spearman correlation.
**Results**: The mean age was 68.66±7.51 years. Grade groups 1–5 comprised 5 (10%), 17 (34%), 10 (20%), 6 (12%), and 12 (24%) cases, respectively. Nineteen cases (38%) had low Ki-67 and 31 (62%) had high Ki-67. The proportion with high Ki-67 increased from 20.0% in grade group 1 to 91.7% in grade group 5. The overall association was significant ($\chi^2$=12.786, df=4, p=0.012), with a positive ordinal relationship (Spearman ρ=0.502, p<0.001).
**Conclusion**: High Ki-67 expression was increasingly frequent across higher ISUP grade groups. Ki-67 may serve as a useful adjunct to morphology for identifying biologically aggressive tumors, although outcome-linked prospective validation and standardized scoring are required.

***Keywords***: *prostate adenocarcinoma; Ki-67; Gleason score; ISUP grade group; immunohistochemistry.*

## INTRODUCTION

Prostate cancer is biologically heterogeneous, ranging from indolent localized disease to rapidly progressive and metastatic malignancy. Histological architecture, summarized by the Gleason score and the five-tier ISUP grade-group system, is therefore a major determinant of prognosis and treatment planning.[1–3]

Grade grouping improves clinical communication, but it cannot capture all intratumoral biological variation. Even tumors assigned to the same grade may have different growth fractions, molecular alterations, treatment responses, and clinical trajectories. Contemporary data also emphasize that individual Gleason patterns retain prognostic information within some high-grade groups.[3,4]

Ki-67 is a nuclear protein expressed during active phases of the cell cycle but absent in quiescent cells. Immunohistochemical measurement of Ki-67 therefore estimates the proliferative fraction of a tumor. In prostate cancer, higher Ki-67 indices have been associated with adverse pathological features, biochemical recurrence, distant metastasis, and disease-specific mortality across surgical and radiotherapy cohorts.[5–10]

The marker is attractive for resource-constrained settings because it can be evaluated on routinely processed paraffin tissue and interpreted with standard light microscopy. Its broader adoption, however, has been limited by heterogeneous thresholds, hot-spot selection, variable counting methods, and intratumoral heterogeneity.[9–12]

Indian institutional data correlating Ki-67 with contemporary ISUP grade groups remain limited. The present study was designed to describe Ki-67 expression in histologically confirmed prostatic adenocarcinoma and test whether a high proliferative index becomes more frequent with increasing grade group.

## MATERIALS AND METHODS

**Study design and setting**: A hospital-based cross-sectional observational study was conducted in the Department of Pathology, Government Medical College and associated hospitals, Kota, Rajasthan. The analysis used 50 consecutive histologically confirmed cases of prostatic adenocarcinoma included in the study during 2022–2024.

**Case selection**: Prostatic core biopsies, transurethral resection specimens, and prostatectomy tissue showing adenocarcinoma on routine histopathology were eligible when adequate viable tumor and clinical data were available. Inadequate or poorly preserved material was excluded. Of the 50 specimens, 44 (88%) were core biopsies, five (10%) were transurethral resections, and one (2%) was a prostatectomy specimen.

**Histopathology**: Formalin-fixed, paraffin-embedded sections were stained with hematoxylin and eosin. Primary and secondary architectural patterns were assigned and combined as a Gleason score. Cases were categorized as grade group 1 (score ≤6), grade group 2 (3+4=7), grade group 3 (4+3=7), grade group 4 (score 8), or grade group 5 (scores 9–10).

**Immunohistochemistry and interpretation**: Sections approximately 5 μm thick were placed on adhesive-coated slides. After deparaffinization, rehydration, heat-induced antigen retrieval, endogenous peroxidase blocking, and incubation with the Ki-67 primary antibody, staining was visualized using the laboratory detection system. Nuclear staining in invasive tumor cells was recorded as the Ki-67 labeling percentage. Expression was classified a priori as low when <10% and high when ≥10% of tumor nuclei stained.

**Statistical analysis**: The association between dichotomized Ki-67 and the five ordered grade groups was assessed by Pearson chi-square testing. Spearman rank correlation evaluated the ordinal relationship between grade group and Ki-67 category. A two-sided $p<0.05$ was considered significant. Calculations were reproduced using Python/SciPy 1.14.1.

**Ethics**: The study was conducted after institutional approval and documented informed consent. Patient identifiers were not used in the article-level analysis.

## RESULTS

The 50 patients were 51–86 years old (mean 68.66±7.51 years). The largest age group was 61–70 years (42%), followed by 71–80 years (34%). Lower urinary tract symptoms were the predominant presentation. Grade group 2 was the most frequent category (34%); 18 cases (36%) belonged to grade groups 4–5.

All tumors demonstrated at least some Ki-67 nuclear labeling. Nineteen cases (38%) were classified as low Ki-67 and 31 (62%) as high Ki-67. High expression was present in 1/5 grade-group 1 cases, 7/17 grade-group 2 cases, 7/10 grade-group 3 cases, 5/6 grade-group 4 cases, and 11/12 grade-group 5 cases.

The distribution differed significantly across grade groups ($\chi^2=12.786$, df=4, $p=0.012$). The increasing frequency of high Ki-67 across the ordered groups produced a moderate positive ordinal association (Spearman $\rho=0.502$, $p<0.001$).

**Table 1. Clinicopathological profile of the study cohort (n=50)**

| Characteristic | Category | n | % |
|---|---|---|---|
| **Age (years)** | ≤60 | 8 | 16 |
| | 61–70 | 21 | 42 |
| | 71–80 | 17 | 34 |
| | >80 | 4 | 8 |
| **Specimen** | Core biopsy | 44 | 88 |
| | TURP | 5 | 10 |
| | Prostatectomy | 1 | 2 |

| | | | |
|---|---|---|---|
| **ISUP grade group** | 1 | 5 | 10 |
| | 2 | 17 | 34 |
| | 3 | 10 | 20 |
| | 4 | 6 | 12 |
| | 5 | 12 | 24 |

**Table 2. Ki-67 category according to ISUP grade group**

| Grade group | Low Ki-67 <10%, n (%) | High Ki-67 ≥10%, n (%) | Total |
|---|---|---|---|
| **1** | 4 (80.0) | 1 (20.0) | 5 |
| **2** | 10 (58.8) | 7 (41.2) | 17 |
| **3** | 3 (30.0) | 7 (70.0) | 10 |
| **4** | 1 (16.7) | 5 (83.3) | 6 |
| **5** | 1 (8.3) | 11 (91.7) | 12 |
| **Total** | 19 (38.0) | 31 (62.0) | 50 |

**Table 3. Recalculated tests of association**

| Analysis | Statistic | p value | Interpretation |
|---|---|---|---|
| **Pearson chi-square** | $\chi^2$=12.786; df=4 | 0.012 | Significant heterogeneity |
| **Spearman ordinal correlation** | ρ=0.502 | <0.001 | Moderate positive relationship |

## DISCUSSION

The present study demonstrated a clear stepwise increase in high Ki-67 expression across ISUP grade groups. Only one fifth of grade-group 1 tumors crossed the 10% threshold, compared with more than nine tenths of grade-group 5 tumors. Both the omnibus comparison and ordinal analysis support the biological premise that loss of glandular differentiation is accompanied by an increasing proliferative fraction.

This finding accords with the foundational observation that Ki-67 identifies cycling cells and with prostate cancer series in which greater labeling correlated with higher grade and adverse outcomes.[5–8] Cowen et al. reported that Ki-67 independently correlated with biochemical failure after radiotherapy, while Pollack et al. found that Ki-67 predicted distant metastasis and mortality among men treated with radiotherapy plus androgen deprivation.[7,8]

Berney et al. also showed prognostic value in conservatively treated localized disease. More recent risk-stratification work continues to evaluate Ki-67 immunoscoring as a method of refining outcome estimates beyond conventional variables.[9]

The association is clinically plausible. Higher-grade tumors have disrupted architecture, genomic instability, and accelerated cycling. A proliferation marker can supply biological information that is not directly represented by glandular pattern alone. In a small biopsy, a high Ki-67 index may also alert the pathologist to aggressive biology when morphological sampling is limited. Nevertheless, Ki-67 should currently be viewed as an adjunct rather than a replacement for complete Gleason pattern and grade-group reporting.

Several methodological concerns affect translation. Prostate cancers are spatially heterogeneous, and hot-spot values can differ from whole-section averages. Mesko et al. quantified substantial intratumoral heterogeneity, illustrating why the field needs standardized selection and counting rules.[10] Automated cell-by-cell analysis and digital pathology may improve reproducibility, but thresholds remain context dependent.[11,12]

The 10% threshold used produced useful discrimination in this cohort, but it should not be interpreted as a universal clinical cutoff. Published studies have used continuous indices, medians, cohort-derived cut points, and different hot-spot methods. Reporting the continuous percentage alongside the prespecified category would be preferable in future work.

The study has several strengths. All cases underwent uniform local processing, the grade-group distribution was available for every case, and the article calculations were independently reconciled. The principal limitations are the single-center design, small sample size, predominance of needle biopsies, absence of interobserver reproducibility assessment, and lack of follow-up for recurrence, metastasis, treatment response, or survival. The cross-sectional association therefore supports biological correlation but cannot establish independent prognostic value.

Interpretation of the effect size is also important. The chi-square result establishes heterogeneity, while the ordinal coefficient indicates that approximately half-rank concordance exists between increasing grade and a high Ki-67 category. The relationship is not perfect: seven grade-group 2 tumors were high, whereas one grade-group 5 tumor remained below 10%. These exceptions are biologically credible and illustrate that histological architecture and proliferative activity

measure related but non-identical features. They also argue against using Ki-67 to revise a grade group without morphological support.

From a reporting perspective, pre-analytical and analytical variables deserve explicit control. Cold ischemia, duration of formalin fixation, block selection, antibody clone and dilution, retrieval conditions, detection chemistry, counterstaining, and the denominator used for counting can all affect the final percentage. Internal positive controls and exclusion of benign glands, lymphocytes, and crushed or necrotic areas are essential. A pathologist should distinguish a true tumor hot spot from entrapped benign proliferative epithelium and should document whether the value represents the highest field, a multi-field mean, or whole-slide analysis.

The distribution in this cohort may partly reflect referral patterns. More than one third of cases were grade groups 4–5, serum PSA was often markedly elevated in the parent dataset, and most tissue came from symptomatic men undergoing biopsy rather than population screening. Consequently, the 62% prevalence of high Ki-67 should not be generalized to all newly diagnosed prostate cancers. The within-cohort association with grade is more interpretable than the absolute prevalence, but even that estimate has wide uncertainty in the smallest groups.

Future prospective studies should use standardized hot-spot counting, report continuous Ki-67 values, include digital quantification where feasible, and relate expression to stage, treatment, biochemical recurrence, metastasis, and survival. Multivariable models are essential to determine whether Ki-67 adds value beyond grade group, PSA, tumor extent, and other recognized factors.

## CONCLUSION

High Ki-67 expression was significantly more frequent in higher ISUP grade groups, rising from 20.0% in grade group 1 to 91.7% in grade group 5. The moderate positive ordinal relationship supports Ki-67 as a practical adjunctive marker of proliferative activity in prostatic adenocarcinoma. Larger outcome-linked studies with standardized scoring are needed before routine prognostic implementation.

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