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ORIGINAL RESEARCH

Evaluation of diagnostic and prognostic value of Ki-67 Immunohistochemistry marker in prostate lesions

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ABSTRACT

Introduction: Prostatic cancer (PC) is one of the most prevalent cancers among men. Early detection is critical because it is only treatable at an early stage. **Aim:** The current study aimed to evaluate Ki-67's diagnostic and prognostic value in prostate lesions, as well as the relationship between Gleason's grade and the degree of Ki-67 expression in PC patients. **Materials and methods:** The present retrospective study was conducted on 109 prostatic biopsies. The paraffin blocks of these prostatic biopsies were retrieved, reprocessed, and re-examined microscopically for various prostatic pathologies. Ki-67 immunohistochemistry staining was performed in all instances. Only cases diagnosed with PC underwent Gleason's grading. The link between Gleason's grade and the degree of Ki-67 expression was analyzed using a Spearman rank correlation analysis. **Results:** Ki-67 expression was found to be negative in 82.61% of BPH cases and positive in 93.55% of PC cases.In 61.54% of well-differentiated PC cases and 58.33% of poorly differentiated PC cases, low positive Ki-67 expression (≤10%) and moderately positive Ki-67 expression (11–30%) were found, respectively.There were no cases with Ki-67 expression of >30% in well-differentiated PC; however, 16.67% of poorly differentiated PC cases had extremely positive Ki-67 expression. **Conclusion:** Ki-67 expression was considerably higher in malignant prostate lesions compared to benign lesions, indicating that Ki-67 as a proliferating marker may be effective in distinguishing PC from BPH.Ki-67 expression was shown to be significantly higher in tumors with a higher Gleason's grade, highlighting its crucial role in the prognosis of PC.

Keywords: Benign prostatic hyperplasia (BPH), Prostate cancer, Gleason grading, Ki-67 immunohistochemistry

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INTRODUCTION

According to the World Health Organization (WHO), prostate cancer (PC)is the secon d most common diagnosed malignancy and the fifth leading cause of death among men worldwide in 2020 [1]. Mortality from prostatic malignancy is strongly related to patient age, with elderly males having a relatively higher incidence. Age, race, and genetic susceptibility are all linked to an increased risk of PC. Because of genetic alterations, PC can range from a slow-growing entity to a rapidly growing tumor with early metastasis [2].Timely diagnosis and management of PChave put huge stress on the global healthcare system.

The management of PC patients with varying degrees of malignancy is a big hurdle, and only active screening can prevent PC from progressing from the early stage to the incurable stage.Early detection of PC has become common since the introduction of screening tests such as the Digital Rectal Examination (DRE) and Prostate Specific Antigen (PSA) test.Even after that, prediction of disease severity with the clinical and histopathological staging is necessary for the selection of therapeutic interventions for patients with PC [3].

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Proliferation is critical in the clinical behaviour of PC, which is significantly linked to tumor aggressiveness and a bad prognosis.However, among the several approaches for assessing proliferation, mitotic counting has been found a reliable and independent predictive value [4]. Ki-67, a proliferative marker, represents tumor cell proliferation rate and is associated with progression, metastasis, and prognosis in a variety of cancers [5,6]. Ki-67 is an antigen that is expressed by the cell nucleus oftumorepithelial cells during the interphase of cell division [7].Several researches have shown that Ki-67 can distinguish between benign and malignant prostate lesions [8,9]. However, its relevance in distinguishing patients at high risk of illness development from the sedentary majority remains debatable.Therefore, the Ki-67 study in PC is needed to know the correlation between Gleason's grading and the intensity of Ki-67 expression.

AIMS AND OBJECTIVES

The present study aimed to assess the diagnostic and prognostic utility of Ki-67 in prostate lesions and to detect the correlation between histopathological Gleason's gradeand intensity of Ki-67 expression in PC patients.

MATERIALS AND METHODS

The current retrospective study was conducted at a tertiary care center in Madhya Pradesh, India, after taking permission from the Institutional Ethical Committee. A total of 109 prostatic biopsies received in the department of pathology were included in the study. Insufficient biopsies and autolyzed specimens were excluded from the present study.Clinical and demographic data, such as age, sex, and presenting complaint, were gathered from the departmental histopathology record registers, as well as the histological diagnosis of various prostatic lesions. The different prostatic biopsies in our histopathological registry were collected through procedures such as transurethral resection of the prostate (TURP) and prostatectomy for histopathological examination (HPE). The paraffin blocks of these prostatic biopsies were retrieved, reprocessed, and the slides were stained with hematoxylin and eosin (H&E) stain. These slides were re-examined microscopically and classified into different prostatic lesions.

Histopathological Gleason's grading was done in all PC cases, and all such cancer cases were divided into 3 groups. Group I consists of Gleason's scores 2-6, which were interpreted as well-differentiated tumors. Group II has a Gleason's score of 7, indicating moderately differentiated tumors, whereas Group III has a Gleason's score of 8–10, indicating poorly differentiated or undifferentiated tumors [10].

Ki-67 staining and interpretation

All paraffin blocks were selected for immunohistochemical (IHC) staining. 3-4 µm thick sections were cut and gently placed on the surface of a 45° C water bath, then wrinkle-free spread onto slides coated with 0.1% poly L-lysine for 15 minutes at 37°C and air-dried. The slides were then baked for 30 minutes on a hot plate at 60°C. Xylene was used to extract the paraffin, which was then rehydrated with degraded ethanol. Antigen retrieval was accomplished by placing slides in a prepared pressure cooker containing citrate buffer, then boiling and left to cool naturally. In a moist environment at room temperature, hydrogen peroxide was introduced to disrupt endogenous enzyme activity.The primary antibody was then introduced to a moist chamber at room temperature for 1-2 hours. Antibody enhancer (super-enhancer) was added to the primary antibody and incubated in a moist chamber for 20 minutes. For secondary staining, the peroxidase anti-peroxidase technique was used. The antigen-antibody combination was colored using DAB (diaminobenzidine). This was followed by hematoxylin counterstaining.

Ki-67 scores were determined by observing sections under a light microscope. The presence of distinct nuclear staining was perceived as a positive. In the area with the highest Ki-67 positivity, 500 cells were counted.The percentage of cells positive for Ki-67 was scored semi-quantitatively, according to the number of labelled cells observed as- Negative = no staining, low positive = ≤10%, moderately positive = 11 to 30%, highly positive = >30% [10].

STATISTICAL ANALYSIS

Medcalc software version 20.027 was used for statistical analysis. The categorical data were expressed using percentages. For categorical data, a chi-square test had been performed, and a Spearman rank correlation analysis was used to produce a correlation analysis. Statistical significance was defined as a p-value of less than 0.05.

RESULTS

The current study was conducted on 109 samples received from prostatic biopsies in the histopathological section. Out of 109 samples, 63.3% (69/109) were diagnosed with benign prostatic hyperplasia (BPH), 8.25% (9/109) were diagnosed withprostatic intraepithelial neoplasia (PIN), and 28.44% (31/109) were diagnosed with PC.The age range 61-70 years represented the majority of cases of BPH (52.17%) and PC (45.16%) [Table/Fig-1].

Out of 31 PC cases, 41.94% (13/31) cases belonged to well-differentiated tumors, while 19.35% (6/31) cases and 38.71% (12/31) cases were interpreted as moderately differentiated and poorly differentiated tumors respectively.

Ki-67 expression was found to be negative in 82.61% (57/69) cases of BPH. Ki-67 expression was found to be positive in the 66.67% (6/9) cases of PIN and 93.55% (29/31) cases of PC. The statistical differences in Ki-67 expression among three groups

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were highly significant with a p-value of <0.0001[Table/Fig-2].

Low positive Ki-67 expression (less than 10%) was found in 61.54% (8/13) of well-differentiated PC cases, whereas moderately positive Ki-67 expression (11-30%) was found in 38.46% (5/13) of well-differentiated PC cases. Among moderately differentiated PC cases, low and moderately positive Ki-67 expression were found to be 83.33% (5/6) and 16.67% (1/6) cases, respectively. 25% (3/12) of cases with poorly differentiated PC cases had low positive Ki-67 expression (≤10%), while 58.33% (7/12) had moderately positive Ki-67 expression (11–30%). No cases were found with Ki-67 expression of > 30% in well and moderately differentiated PC, while 16.67% (2/12) of poorly differentiated PC cases were found to have highly positive Ki-67 expression (>30%) [Table/Fig-3].Correlation analysis showed a moderate positive correlation between Ki-67 expression and Gleason's score in PC cases, with correlation coefficient Rs=0.5875, p=0.002 [Table/Fig-4].This indicated that a higher Gleason's score is associated with higher Ki-67 expression.

[Table/Fig-1]: Distribution of different prostatic lesion cases according to age group

Age (in Years)	BPH*	PIN#	PC$
<50	6 (8.69%)	1 (11.11%)	2 (2.56%)
51-60	17 (24.63%)	4 (44.45%)	7 (22.58%)
61-70	36 (52.17%)	3 (33.33%)	14 (45.16%)
>70	10 (14.49%)	1 (11.11%)	8 (25.8%)
Total	69 (100%)	9 (100%)	31 (100%)

Benign prostatic hyperplasia, #Prostatic intraepithelial neoplasia, $Prostate cancer

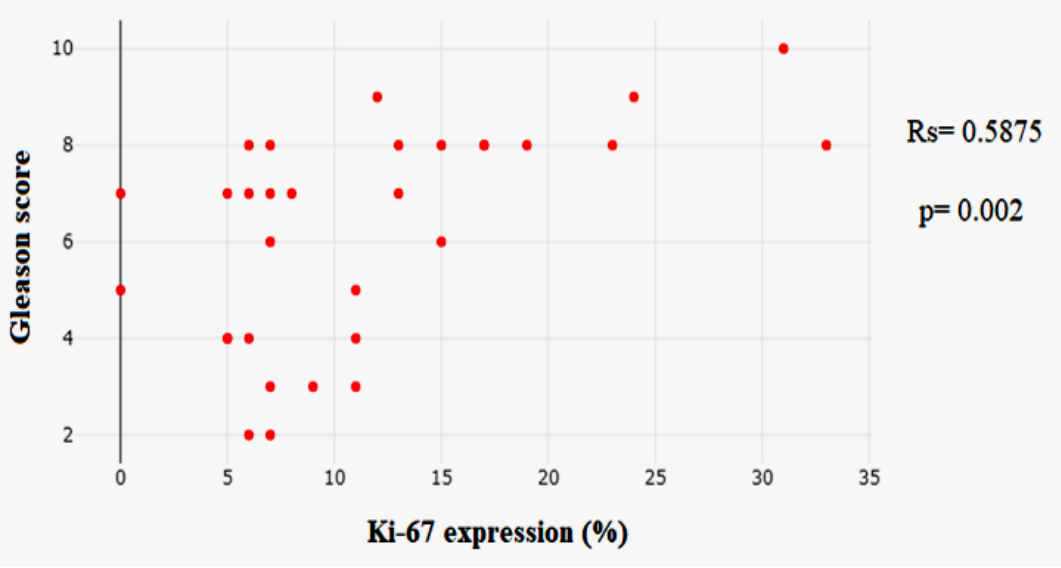
[Table/Fig-2]:Distribution of Ki-67 expression in different prostatic lesions (n=109)

Prostatic lesions	Ki-67 expression		Total	Statistical analysis!
	Negative	Positive		
BPH*	57 (82.61%)	12 (17.39%)	69 (100%)	□^{2} =52.799 p <0.0001 Statistically significant
PIN#	3 (33.33%)	6 (66.67%)	9 (100%)	
PC$	2 (6.45%)	29 (93.55%)	31 (100%)	

*! Statistical analysis of data was done by using chi-square test. P value less than 0.05 was considerd significant. *Benign prostatic hyperplasia, #Prostatic intraepithelial neoplasia, $Prostate cancer*

[Table/Fig-3]: Distribution of intensity of Ki-67 expression among prostatic cancer cases (n=31)

Prostatic cancer cases (n=31)	Ki-67 expression			Total
	≤ 10%	11-30%	>30%	
Well differentiated	8 (61.54%)	5 (38.46%)	0	13 (100%)
Moderately differentiated	5 (83.33%)	1 (16.67%)	0	6 (100%)
Poorly differentiated	3 (25%)	7 (58.33%)	2 (16.67%)	12 (100%)



[Table/Fig-4]:Spearman rank correlation analysis between intensity of Ki-67 expression and Gleason's score in prostatic cancer cases (n=31)

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DISCUSSION

Prostatic lesions affect men in their later years, ranging from benign to malignant, and require early detection because PCis a rapidly growing tumor [11].Our study showed that most of the prostatic lesions were benign (63.3%), when compared to premalignant (8.25%) and malignant (28.44%) lesions.Padmapriya BS et al.,[12] and Shekhar S et al.,[13]found a higher incidence of benign prostatic lesions than premalignant and malignant lesions, which is similar to our findings.The current study found that prostatic lesions became more common as people became older. The sixth decade had the highest number of instances; Kumari K et al.,[14]and Gangwar S et al.,[15]found similar results, with the sixth decade having the highest number of cases.

Many researchers used the Ki-67 protein to assess the proliferation rate, which has been shown to be an unfavourable prognostic marker in breast cancer [6,16,17]**.**Uncontrolled proliferation is a hallmark of cancer, and IHC evaluation of Ki-67 protein is the most extensively used assessment of a tumor's proliferation potential [18].In the current study, we found that Ki-67 staining was negative in 82.61% of BPH cases and positive in 93.55% of PC[Table/Fig-2].This finding is in-line with the study done by Bakna M et al.,[8].Ki-67 is a proliferative biomarker that reveals the proliferation of tumor cells that express it, and it is thought to be linked to the tumor proliferation index's aggressiveness [18].This explains why low Ki-67 expression was seen in BPH cases, as BPH is a condition linked with decreased apoptosis rather than increased proliferation . Positive Ki-67 staining, on the other hand, we found in cases with PC independent of tumor grade.

Ki-67 expression was found to be substantially linked with bone and lymph node metastases of PC in a study by Song JQ et al.,[10]. A higher Gleason’s score was linked to a higher chance of prostatic cancer spreading to the bone and lymph nodes, according to him [10].Previous researchers reported that poorly differentiated prostatic tumors had a high incidence of Ki-67 expression as compared to well-differentiated prostate tumors [19,20].In our study, we found that the intensity of Ki-67 expression was different according to the differentiation of prostate tumors.The intensity of Ki-67 expression was low in well-differentiated prostate tumors, while the intensity of Ki-67 expression was high in poorly differentiated tumors[Table/Fig-3].This finding is in line with the study done by Gangwar S et al.,[15].An increasing proliferation rate seems to promote a lower degree of differentiation in cancer cells. That’s why Ki-67 expression is increased in poorly differentiated PC because Ki-67 is a proliferative biomarker.

In their study, Verhoven B et al.,[21] found that a high proliferative index in prostate cancer is linked to radiation failure. They also observed that higher Ki-67 levels were associated with a shorter survival time in patients with the metastatic illness. In their study, Hammarsten Pet al.,[22]found that higher Ki-67 expression was associated with an advanced clinical stage of prostate cancer. Munoz E et al.,[23] did not find a significant link between Ki-67 and Gleason's score, contrary to our findings.However, research conducted by Urs RAN et al.,[19], Madani SH et al.,[20], and Murti K et al.,[24] found a statistically significant association between the Ki-67 index and Gleason's score of tumors with low- to high-grade differentiation, as well as an essential relationship with the prognosis of PC. In cases of PC, our research found a moderate positive link between Ki-67 expression and Gleason’s score, with a correlation coefficient of Rs=0.5875, p=0.002 [Table/Fig-4].This meant that a higher Gleason’s score was linked to a higher Ki-67 expression.Ki-67 expression rises with the Gleason’s score, indicating the importance of proliferation in cancer development and progression. Thus, Ki-67 expression acts as an indicator that aids in the identification of tumors with a high rate of cell growth and, as a result, is a good prognostic marker.

LIMITATION(S)

The present study has a few important limitations. The current investigation was conducted in a single center. As a result, the study's conclusions cannot be extrapolated to the broader population.More experiments are needed to verify the accuracy of the experimental results due to the temporal and histological heterogeneity of PC.There is still much to know about malignant prostate tumors. Although there are numerous indicators for tumor detection, only one was used in this study.As the technology advances, in-depth research on the various detection indicators will need to be conducted to assess the diagnosis and prognosis of PC.

CONCLUSION(S)

Our study revealed that Ki-67 expression was significantly high in malignant prostate lesions as compared to benign lesions, indicating that Ki-67 as a marker of proliferation is very reliable and may be useful in distinguishing prostate cancer from BPH. Ki-67 expression was constitutively active in prostatic carcinomas, and increased expression was typically observed in high-grade malignant prostatic tumors. Ki-67 expression was found to be significantly higher in tumors with a higher Gleason’s grade in the current study, indicating its critical role in the prognosis of these cancers. Conclusively, the IHC approach can simply measure Ki-67 expression, and it might be used as a supplement diagnostic and prognostic tool in addition to traditional prognostic and predictive indicators like serum prostate specific antigen (PSA) level, clinical staging and Gleason’s score in everyday practice.

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