

## Ki-67 Immunohistochemical Expression in Prostatic Lesions

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### Abstract

<b>Background</b>	The cell proliferation marker, ki-67, is a nuclear and nucleolar protein, which can be detected during all active phases of the cell cycle (G1, S, G2, and mitosis), but absent from cell resting phases. Thus, it is considered as an excellent marker for determination of the cell growth fraction and it has been detected to be a useful marker in predicting the development of human tumors.
<b>Objective</b>	To evaluate the immunohistochemical expression of the antigen ki-67 in benign, pre-malignant and malignant prostatic lesions.
<b>Methods</b>	A cross section study of 115 paraffin embedded prostatic tissue blocks, 76 cases were benign prostatic hyperplasia (BPH), 9 cases were high grade-prostatic intraepithelial neoplasia (HG-PIN), and 30 cases were prostatic carcinoma (PCa). Sections from each block were prepared for immunohistochemical staining of ki-67.
<b>Results</b>	Ki-67, semi-quantitative evaluation, revealed that the majority of BPH (88.2%) and HG-PIN (66.7%) presented weak positivity (+), on the other hand, the majority of PCa (60.0%) presented moderate positivity (++) and 16.7% showed intense positivity (+++). For prostatic carcinoma (PCa), no significant association was found between Ki-67 and serum tPSA level, while a significant association with Gleason grade was found, the higher grade ( $\geq 7$ ), the more intense positive immunolabeling for Ki-67.
<b>Conclusion</b>	Significant differences between ki-67 immunolabeling and histological type of prostatic lesions, between BPH and HG-PIN, and prostatic carcinoma, which may have potential to evolve to malignancy.
<b>Keywords</b>	BPH, HG-PIN, Ki-67, prostatic carcinoma, tPSA
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**List of abbreviations:** BPH = Benign prostatic hyperplasia, HG-PIN = High-grade prostatic intraepithelial neoplasia, PCa = Prostatic carcinoma, PSA = Prostatic specific antigen

### Introduction

The prostatic pathology is a common condition in the worldwide, and cause a considerable disability in elderly men, specially lower urinary tract symptoms, in form of urine retention, dripping, hesitancy, and others<sup>(1-3)</sup>. The prostatic glandular tissue starts proliferating after 40 years of age in 50% of

men, and 80% by 70 years<sup>(3,4)</sup>. The proliferating prostatic tissue compresses the prostatic urethra, which lead to significant considerable disability with difficulty in passing the urine. The condition called benign prostatic hyperplasia (BPH)<sup>(4-6)</sup>.

The prostate cancer is a common disease of elderly men worldwide, and ranks the fifth solid, non-cutaneous, non-hematological cancer in Iraq<sup>(7)</sup>, it can be slow-growing, and identified in asymptomatic patients. Most of

the malignancies of the prostate arise from the glandular tissue, thus, the commonest type is adenocarcinoma<sup>(8,9)</sup>. About 70% of prostate cancer rise from the peripheral zone, 15-20%, and 10-15% arise in the central zone and transitional zone respectively<sup>(9-11)</sup>. Most of prostatic carcinoma are multifocal, with involvement of multiple zones<sup>(12,13)</sup>.

Most of the patients with prostatic carcinoma are identified by screening in asymptomatic men, by assessment of serum level of prostatic specific antigen (PSA) and digital rectal examination<sup>(14,15)</sup>. In addition, prostatic cancer can be an incidental pathological finding when surgically removed to relieve obstructive urinary symptoms from BPH<sup>(15)</sup>.

Intraepithelial proliferative lesions, an important lesion, is high-grade prostatic intraepithelial neoplasia (HG-PIN) which considered as premalignant condition for prostatic adenocarcinoma may identified incidentally by tissue biopsy, in which risk of malignant transformation ranging from 9-30%<sup>(15,16)</sup>, or may coexist with underlying prostatic adenocarcinoma. Clinically, HG-PIN is not associated with elevated serum PSA level, and don't produce abnormality in the prostatic texture or sized by digital rectal examination, unless prostatic carcinoma coexisted<sup>(16,17)</sup>. Early detection of HG-PIN lesions and follow up of the patients by 3-6 months' interval<sup>(16-18)</sup>, by prostatic needle biopsy can contribute to eradicate early prostatic adenocarcinoma.

The nuclear expression of the Ki-67 protein is associated with cell proliferation, as it is present during all active phases of cell cycle (G1, S, G2, and mitosis), but absent from resting cells<sup>(19,20)</sup>. Therefore, Ki-67 protein is considered as an excellent marker for cell proliferation (growth fraction), and it is associated with a high mitotic count and high histological grade<sup>(21,22)</sup>.

The objectives of this study were to evaluate the immunoexpression of the antigen ki-67 in benign, pre-malignant and malignant prostatic lesions, and distinguish the relation between ki-67 immunoexpression and serum tPSA level

and Gleason grade for cases of prostatic carcinoma.

### Methods

This was a cross sectional study approved by Institute Review Board of College of Medicine, Al-Nahrain University. The collection of samples last for the period from March, 2015 to February 2016, a total of 115 formalin fixed paraffin embedded prostatic tissue of which (76) cases were BPH, (9) cases were HG-PIN, and (30) of prostatic adenocarcinoma, were retrieved from the histopathology archive of Teaching Laboratory in Medical City, for the period from 2013 to February 2016.

All the clinicopathological parameters, which included age of the patient, preoperative total serum PSA, histopathological type and Gleason grade for cases of prostatic carcinoma, were obtained from patient's admission case sheets and pathological reports. Any sample lacking the clinicopathological information was excluded from this study.

For each case, one representative (4 µm) section was stained with Hematoxylin and Eosin, and histopathological diagnosis was revised, other 4 µm section was placed on positively charged slide and stained immunohistochemically using three steps-indirect streptavidin method for monoclonal mouse antibodies including monoclonal mouse, anti-human, Ki-67 antigen, manufactured by Dako.

### Interpretation of the results of immunohistochemical staining

Staining results of Ki-67: brown nuclear stain is considered positive. The percentage of cells positive for Ki-67 was scored semi-quantitatively, according to the number of stained cells observed as:

- Weak (marked as +), <25%
- Moderate (marked as ++), 25-75%
- Intense (marked as +++), >75%

Statistical analysis was performed with SPSS V.16, using chi-square, t-test with p value of <0.05.

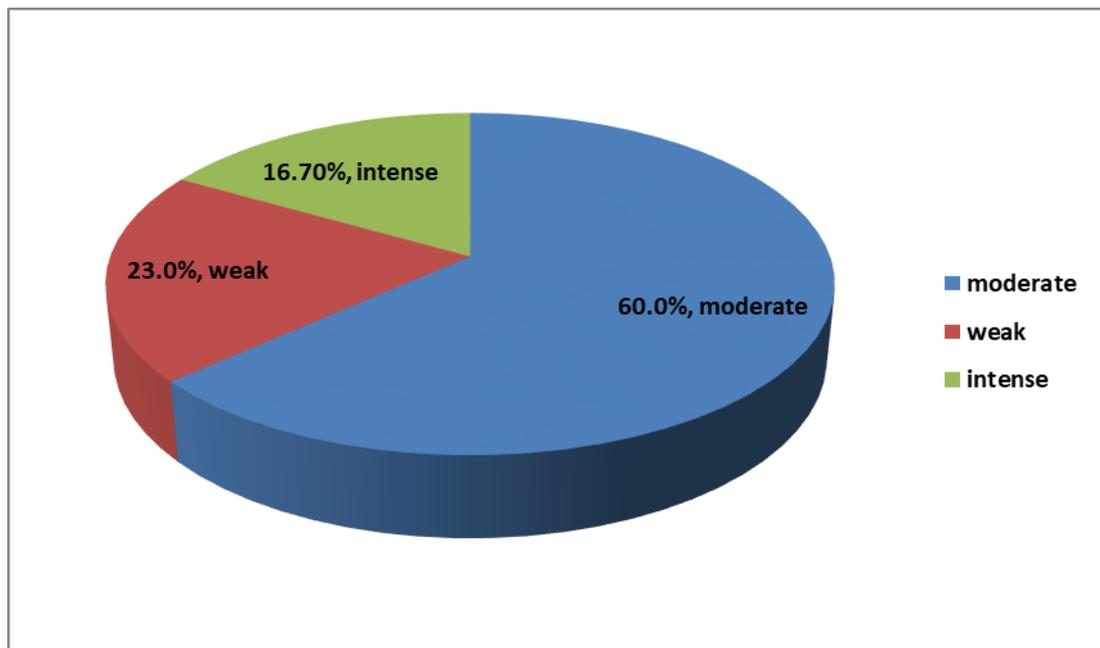
## Results

Semi-quantitative evaluation of the immunolabeling for ki-67 demonstrated that the majority of the BPH (88.2 %), and GH-PIN (66.7%) presented weak positivity (+), (Table 1). On other hand, the majority of prostatic

carcinoma (PCa); (60.0%) presented moderate positivity (++) , while intense positivity (+++) and weak positivity (+) were found in 16.7% and 23.3% of the cases respectively (Figure 1).

**Table 1. Results of ki-67 immunoexpression among presented cases of paticular prostatic pathology**

Ki-67 expression	BPH		HG-PIN		PCa	
	No.	%	No.	%	No.	%
Negative	5.0	6.6	1.0	11.1	0.0	0.0
Positive (+)	67.0	88.2	6.0	66.7	7.0	23.3
Positive(++)	3.0	3.9	2.0	22.2	18.0	60.0
Positive(+++)	1.0	1.3	0.0	0.0	5.0	16.7
Total	76.0		9.0		30.0	



**Figure 1. Ki-67 expression among presented cases of PCa**

As shown in table (2), there was no significant association between Ki-67 positivity and serum total PSA levels, ( $p=0.32$ ).

Regarding Gleason grade, there was a significant association between Ki-67

immunolabeling and higher grade. As shown in table (3), intense positive ki-67 immunoexpression was higher (25%) in grade 7 and lowest in grade 6 (zero%), ( $p=0.039$ ).

**Table 2. Ki-67 immunolebeling correlated to mean age and mean total serum PSA among presented cases of PCa**

Ki-67 immunoexpression	Mean total S.PSA level (ng/ml)
Weak (+)	12.1±1.2
Moderate (++)	10.5 ±1.4
Intense (+++)	10.9±1.5

No significant association, p=0.32

**Table 3. Ki-67 immunolabeling correlated to Gleason grade among presented cases of PCa**

Gleason score	No.	Weak (+)		Moderate (++)		Intense (+++)	
		No.	%	No.	%	No.	%
6	4	2.0	50.0	2.0	50.0	0.0	0.0
7	8	1.0	12.5	5.0	62.5	2.0	25.0
8	10	2.0	20.0	6.0	60.0	2.0	20.0
9	8	2.0	25.0	5.0	62.5	1.0	12.5
Total	30.0	7.0		18.0		5.0	

Significant association, p=0.0276

### Discussion

For current study, it has been showed that Ki-67 weakly expressed in majority of BPH and HG-PIN (88.2% and 66.7%, respectively). For prostatic adenocarcinoma, the majority of the cases showed moderate positivity (60%), intense positivity was found in 16.7% of cases. No significant association was found between serum tPSA level and Ki-67 immunoexpression. Gleason score ( $\geq 7$ ) were found to be associated with intense Ki-67 expression.

Mucci et al. in 2000 have demonstrated statistically significant increases in the expression of Ki-67 were seen from normal tissue to HG-PIN to prostatic carcinoma <sup>(23)</sup>.

Munoz et al. in 2003, in a semi-quantative evaluation of Ki-67 immunolabeling in prostatic lesions, demonstrated that the majority of the BPH lesions (85.7%), and GH-PIN (72.0%), presented with weak positivity (+). On other hand, the majority of the PCa (62.9%), presented moderate positivity, with a significant correlation between Ki-67 immunolabeling and histological diagnosis. There were highly significant differences in ki-67 expression between BPH and PCa, and HG-PIN and PCa, with significant differences being

found between Gleason grades and Ki-67 immunoreactivity. The immnoreactivity for ki-67 increase in accordance with the increase of the grade of histological lesion and the greater immunoreactivity being found in high Gleason grade <sup>(24)</sup>.

Zhong et al. in 2008 revealed increased Ki-67 immunoexpression in prostatic carcinoma and BPH (P<0.05), relative to human normal prostatic tissues <sup>(25)</sup>.

Sulik et al. in 2011 reported a significant association between Ki-67 expression in prostatic carcinoma and Gleason score ( $\geq 7$ ), but no association with pre-operative PSA level <sup>(26)</sup>.

Verma et al. in 2015 have found Ki-67 expression in 64% of the cases. All cases of well differentiated PCa (low Gleason grade) lack Ki-67 expression, while moderately and poorly differentiated PCa had showed ki-67 immunostaining. A significant correlation was found between Ki-67 positivity and increased Gleason's grade, the higher Gleason grade, the higher ki-67 immunoreactivity <sup>(27)</sup>.

Kaur et al. in 2016 demonstrated a significant association between ki-67 immunoexpression

and higher Gleason grade in prostatic carcinoma ( $\geq 7$ )<sup>(28)</sup>.

Rajeswari in 2016 found a significant association between ki-67 immunostaining and Gleason grade, and can be used as a prognostic marker<sup>(29)</sup>.

These studies support the findings of the present study that Ki-67 immunostaining significantly differs between benign, pre-malignant and malignant prostatic lesions, with a significant association of ki-67 expression and higher Gleason grade ( $\geq 7$ ) in prostatic carcinoma.

This study concluded that there are significant differences between ki-67 immunostaining and histological type of prostatic lesions, between BPH, HG-PIN, and prostatic carcinoma, which may have potential to evolve to malignancy. Also, Ki-67 protein immunolabeling is significantly associated with Gleason grade, but no significant association had been found with serum tPSA level.

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### Author contributions:

All authors contributed to this manuscript. They coordinated study subject recruitment, implementation and progress of this study, and helped with data interpretation and manuscript organization and editing.

### Conflict of interest

All authors have no conflict of interest.

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