



Ki-67 IMMUNOEXPRESSION IN ADENOCARCINOMA OF PROSTATE AND ITS CORRELATION WITH GLEASON GRADE

Pathology

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ABSTRACT

Background: Prostate cancer represents the second most frequently diagnosed malignancy among the male and fifth leading cause of death. Tumour grade is one of the most important prognostic factors of prostate cancer. There are many markers studied in prostatic cancers, but the most extensively studied one is Ki-67, the proliferative marker. Prostatic cancers with Ki-67 over expression are generally aggressive. **Aim And Objectives:** - Aim of this study is evaluate ki67 immunoexpression in adenocarcinoma of prostate and to correlate with histopathological grade (Gleason Grade). **Material Methods:-** This is single centre cross sectional study. The patient who visited department of urology and renal transplant in Gauhati medical college and hospital and whose TRUS guided biopsy were received in the pathology department and diagnosed as adenocarcinoma were included. A total 50 cases were analysed. Tumour grade was determined according to Gleason's grading system and Ki-67 expressions were determined by IHC staining. The obtained results were analysed and evaluated using Chi square test (IBM SPSS version 21). **Result And Interpretation:-** A statically significant correlation found between Gleason Grade and Ki-67 immuno expression with a p value of <0.001. **Conclusion:-** In this study a statistically significant correlation was found between Gleason Grade and ki-67 labelling index. Hence Ki-67 immunoexpression evaluation and its result can be incorporated with histopathology which may influence clinical decision making as prognosis of prostate cancer.

KEYWORDS

Prostate adenocarcinoma, Gleason Grade, Ki-67 labelling index

INTRODUCTION

Prostate cancer is a major health problem being second most frequently diagnosed cancer and fifth leading cause of cancer death in males¹. Incidence of carcinoma prostate increases proportionally after 50 years of age² More than 95% prostate cancer are adenocarcinoma.³ The most powerful histopathological predictor being Gleason's grading system.⁴ Ki-67 recognizes a proliferation specific nuclear antigen. This antigen is expressed in proliferating cells in late G1, S, G2 and metaphase, but does not in resting cell G0.⁵

It has been found that ki67 LI is higher in Prostate carcinoma than BPH and is more in metastatic PCa than non-metastatic PCa. Ki-67 labelling index determines clinical behavior and aggressiveness of tumor. It is found that prostate cancer with high Ki-67 LI shows faster cellular growth and behave aggressively.⁵

Aim of this present study is carried is to identify expression Ki-67 labelling index adenocarcinoma of prostate and to correlate with histological Gleason grade.

MATERIAL AND METHODS

This is a hospital based cross sectional study of 50 prostatic adenocarcinoma cases diagnosed in TRUS (Transrectal ultrasonography) guided biopsy in a tertiary care centre of north east for a duration of one year between July, 2021 to June, 2022. All TRUS (Transrectal ultrasonography) guided biopsy detected adenocarcinoma of prostate cases who have given consent were included in this study. Other metastatic carcinoma, benign lesion was excluded from this study.

Brief clinical data including history, pretreatment serum PSA, DRE findings were collected from Urology Department.

Proper processing of biopsy tissue was done. paraffin sections were stained by Hematoxylin and eosin stain. Adenocarcinoma cases were histologically graded according to Gleason's grading system and Modified Gleason's grading system [ISUP grade].

The cases diagnosed as adenocarcinoma and graded by Gleason grading system were stained immunohistochemically by anti Ki-67

antibody for Ki-67 labelling index .

The immunoquantification was performed using percentage of tumour cells that react with the antibody. Each slide was evaluated at 40X magnification in order to find areas with maximum positive cells. Then these areas were examined at 400X magnification and the percentage of positive cells to total cells was calculated. At least 500 cells were counted, and only the cells that were positive for the desired marker were considered.

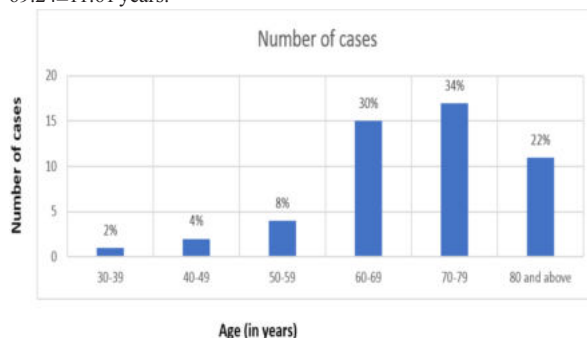
Tumours were divided into five groups according to the percentage of Ki-67 positive cells. Cases in which the percentage of stained cells was $\leq 2\%$ were considered negative. Cases with Ki-67 index of $\leq 25\%$ were considered 1+, 26–50% as 2+, 51–75% as 3+ and 76–100% as 4+.¹⁰

The results were analyzed using IBM SSPS Soft were V21. For correlation of ki67 labelling index with Gleason grade a P value of <0.05 was taken as statistically significant. The study protocol was approved by the institutional ethics committee.

RESULT AND OBSERVATION

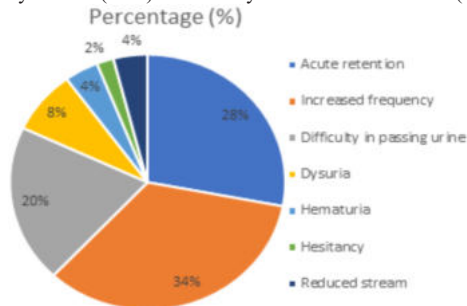
50 cases of adenocarcinoma of prostate were included in this study.

The age of the patient ranged from 37 to 87 years with mean age 69.24 $\pm$ 11.61 years.



Graph 1:- Distribution of cases according to age groups

All the patient presented to urology OPD with lower urinary tract symptoms (LUTS). The most predominant LUTS symptoms increased frequency of urine (38%) followed by acute retention of urine (28%).



Graph 2:- Distribution Of Cases Based On Clinical Feature

- The mean PSA level 77.38 ± 49.14 ng/ml. The maximum value of PSA is 200 ng/ml and minimum PSA value is 8 ng/ml.
- Out of 50 cases 38 (76%) cases showed abnormal digital rectal examination.

Table 1. Distribution Of Cases According To Total Gleason Score

Total Gleason score	No of cases	Percentage
6	5	10%
7	33	66%
8	5	10%
9	5	10%
10	2	4%
Total	50	100%

Out of 50 adenocarcinoma cases Gleason score 6 was seen in 5(10%) cases, score 7 was seen in 33(66%) cases, score 8 was seen in 5(10%) cases, score 9 in 5(10%) cases and highest Gleason score 10 was seen in 2 (4%) cases.

Table 2. Distribution Of Cases According To Gleason Grade Group

Gleason grade group	No of cases	Percentage
1	5	10%
2	11	22%
3	22	44%
4	5	10%
5	7	14%
Total	50	100%

Out of 50 cases grade group 1 was seen in 5(10%) cases, grade group 2 was seen in 11(22%) cases, grade group 3 in 22(44%) cases, grade group 4 in 5(10%) cases and highest Gleason grade 5 was seen in 7(14%) cases.

Table 3. Distribution Of Cases According To Ki-67 Labelling Index

Ki-67 labelling index	No of cases	Percentage
negative	11	22%
1+(3-25%)	21	42%
2+(26-50%)	10	20%
3+(51-75%)	7	14%
4+(76-100%)	1	2%
Total	50	100%

Majority of cases 42% had 1+ positivity, 22 % cases were negative, 20% cases showed 2+ positivity, 14% cases showed 3+ positivity and only 2% cases showed 4+ positivity.

Microphotograph

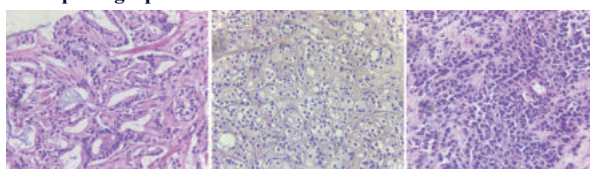


Fig1: Microphotograph of Gleason pattern 3 (well-formed glands) (40x10, H&E)

Fig2: Microphotograph of Gleason pattern 4 (fused glands) (40x10, H&E)

Fig 3:- Microphotograph of Gleason pattern 5 (cells are arranged in sheets) (40x10, H&E)

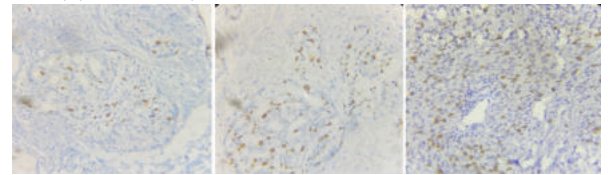


Fig4: 1+ Ki-67 labelling index (40x10)

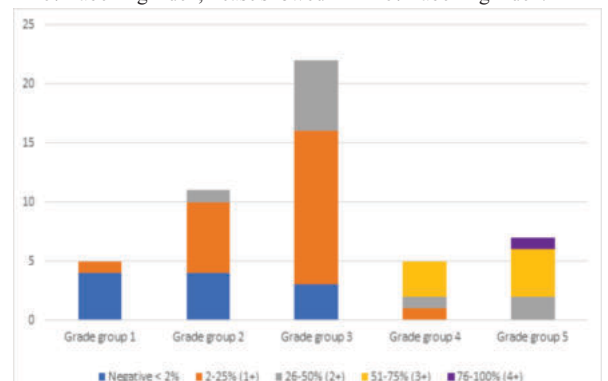
Fig5: - 2+ Ki-67 labelling index (40x10)

Fig6: - 3+ Ki-67 labelling index (40x10)

Table 4. Distribution of cases according to Ki67 labelling index in relation to Gleason grade

Ki-67 labelling index	Gleason grade group 1	Gleason grade group 2	Gleason grade group 3	Gleason grade group 4	Gleason grade group 5	Total	P value
negative	4	4	3	0	0	11	<0.001
1+	1	6	13	1	0	21	
2+	0	1	6	1	2	10	
3+	0	0	0	3	4	7	
4+	0	0	0	0	1	1	
Total	5	11	22	5	7	50	

Out of 5 cases of grade group 1, 4 (8%) cases were negative, 1 case showed 1+ Ki-67 labelling index. Out of 11 cases of grade group 2, 4 cases (8%) were negative, 6 cases (12%) were 1+ Ki-67 Labelling Index, 1 Case(2%) showed 2+ Ki-67 Labelling Index. Out of 22 cases of Grade group 3, 3 cases (6%) were negative, 13 cases (26%) showed 1+ Ki-67 Labelling Index, 6 cases (12%) showed 2+ Ki-67 Labelling Index. Out of 5 cases of Grade group 4, 1 case (2%) showed 1+ Ki-67 Labelling index, 1 case (2%) showed 2+ Ki-67 Labelling Index, 3 cases (6%) showed 3+ Ki-67 Labelling Index. Out of 7 cases of Grade group 5, 2 cases (4%) showed 2+ Labelling index, 4 cases showed 3+ Ki-67 Labelling index, 1 case showed 4+ Ki-67 Labelling Index.



Graph 3: - Distribution Of cases based on Gleason grade group and Ki67 Labelling index

A statistically significant correlation was found between Gleason grade group and Ki67 Immunoeexpression with a P value of <0.001.

DISCUSSION

Prostate cancer is the most common solid neoplasm among elderly male. Carcinoma prostate constituted the second commonest group disease next to BPH. Now a days disease is diagnosed earlier because of combined use of digital rectal examination, serum PSA measurement, multiparametric MRI (MpMRI), biopsy.

In the present study all the patients complained of LUTS with frequency of urine as predominant symptoms followed by acute retention of urine. Kirokaya B et al¹⁵ and Chouhan et al²⁰ also found increase frequency of urine as common symptoms which was similar to our study.

In the present study it is found that adenocarcinoma of prostate occurs mostly in elderly male with mean age of the patient 69.24 ± 11.61 years ranging from 37 to 87 years. This is comparable with the study done by CA Okolo et al¹⁷ and Elijah et al¹⁸.

DRE is the most sensitive method for diagnosis of palpable prostatic abnormalities; however, it lacks specificity for prostatic malignancy. In this present study it is demonstrated that 76% cases had abnormal digital rectal examination which is comparable to the study conducted by Ojewala et al¹⁹.

In our study Gleason score 7(66%) is the predominant pattern which comparable with study conducted by Gopinath Barui et al³⁰.

Although the reproducibility is not perfect, still the Gleason's grading system is the most favoured prognostic factor, and highly significantly associated with survival and/or progression.

Table 5. Different percentage of Gleason grade group in different studies

Gleason Grade	Studies					
	Aihara et al ²¹ (1994)	Murtala Abubakkar et al ²⁰ (2008)	Gopinath Barui et al ²⁰ (2019)	Mohammad et al ²⁴ (2020)	Pooran Priya et al ²³ (2020)	Present study
1	0%	32.2%	0%	27.2%	5.8%	10%
2	1%	6.2%	17.41%	6.71%	0%	22%
3	84%	14%	34.78%	17.8%	29.4%	44%
4	5%	29.9%	34.78%	38.1%	11.8%	10%
5	0%	18%	13.04%	10.2%	58.8%	14%

The findings of the present study regarding Gleason grade group is comparable with studies conducted by Gopinath Barui et al²⁰, Mohammad et al²⁴.

In addition to Gleason's grading, assessment of proliferative status by Ki-67 is a widely accepted fact.

Table 6. Different percentage of Ki-67 labelling index in different studies

Study	year	Ki-67 labelling index				
		<2%	3-25%	26-50%	51-75%	76-100%
Munoz et al ²⁵	2003	0	22.8%	62.9%		14.8%
Madani et al ⁶	2011	28.5%	48.9%	16.3%		6.3%
Rasheed et al ¹⁰	2020	0%	23.3%	60%		16.7%
Present study	2022	22%	42%	20%	14%	2%

In this study a Statistically significant correlation is found between modified Gleason grade and ki67 labelling index with a p value <0.001.

Correlation of Ki-67 Immuno expression with modified Gleason grade in different studies

Similar to our study various studies across the globe found that there is a statistically significant correlation of Ki-67 immunoexpression with modified Gleason grade.

Table 7

S L No	studies	Year	No of cases studied	Relationship of Ki-67 Labelling Index with Gleason grade
1	Madani et al ⁶	2011	49	Statistically significant association
2	Sulik et al ⁵	2011	56	Significant association P value<0.04
3	Shanemesko et al ¹¹	2013	77	Statistically significant correlation p=0.01
4	Rajeswari k et al ⁸	2015	46	Statistically significant association p value < 0.05
5	Harjout kaur et al ⁹	2016	50	No statistically significant correlation
6	Rasheed et al ¹⁰	2017	30	Significant association p value=0.039
7	Roopa Urs Et al ¹¹	2018	50	No significant association p value =0.277
8	Mohammad et al ¹²	2018	47	Significant correlation (p value <0.05)
9	Summaitra Siddique et al ¹³	2021	50	Significant correlation (p value<0.001)
10	Miranti et al ¹⁴	2021	31	Significant correlation (p =0.013)
	Present study	2022	50	Significant correlation p<0.001

CONCLUSION:-

Histopathological diagnosis and Gleason's grading plays a definitive role in the management of prostatic cancer. Ki-67 expression, a cell proliferation marker is significantly up-regulated in malignant lesion and show an increase in the expression of this marker with increasing grade and score. In present study a statistically significant correlation was found between Gleason grade group and Ki-67 labelling index with a P value of <0.001 which implies that Ki-67 is a useful marker to assess the aggressiveness of neoplastic lesion mainly prostate adenocarcinoma.

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