



Original Article

Expression of Ki-67 in Prostate cancers and its correlation with Histopathological Grade and serum Prostate-specific antigen (PSA) levels: A study from eastern part of Uttar Pradesh

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Abstract

Background: *One of the commonest causes of deaths among men is prostate cancer. The biological behavior of these tumors is associated with their degree of cellular proliferation. Assessment of over-expression of Ki-67 labeling index is an objective measurement of proliferation and its association with Gleason grading can describe the aggressiveness of these cancers. Thus, the present study was undertaken to study the correlation between Ki-67 expression and histopathological grade with serum PSA levels.*

Methods: *Twenty seven cases of histopathologically proven prostate cancers were studied over a period of 1.5 years and Gleason grade was assigned in each case. Expression of Ki-67 was analyzed using immunohistochemistry.*

Results: *Of the total 27 cases, Ki-67 was expressed in majority of the moderately and poorly differentiated tumors with 18 cases (66.6%) showing strong positivity. 8 cases (29.62%) revealed serum PSA levels above 20 ng/ml. Gleason's score of more than 7 was seen among 25 cases (92.6%). There was no significant ($p>0.05$) association of PSA level with Ki-67. However, association of Ki-67 index and Gleason's Grade was significant ($p=0.03$).*

Conclusion: *Proliferative activity determined by Ki-67 reflects the aggressive behaviour of prostate cancers and association with Gleason's grade can aid in predicting the recurrence and deciding the appropriate therapy.*

Keywords: *Adenocarcinoma Prostate; Ki-67; PSA; Gleason's Grade; Prognosis.*

Introduction

Prostate cancer is the second most common cause of cancer and presently the sixth leading cause of cancer death among men all over the world. Mortality associated with prostatic carcinoma is strongly related to the age of patient with relatively higher incidence observed among elderly males. With the advent of screening tests like Digital Rectal Examination (DRE) and Prostate Specific Antigen (PSA) test, early detection in younger population has become frequent.¹

Cellular proliferation is the hallmark of these cancers and determines their clinical behaviour. Ki-67 is an antigen expressed by cell nucleus when the cells are dividing themselves. It is widely used to assess the tumor proliferation rate. Numerous studies have revealed that increased Ki-67 index in these tumors strongly correlates with the tumor aggressiveness and poor prognosis.² Although its role as an independent prognostic marker is still controversial, correlation with histopathological grade and other clinical parameters including serum PSA levels can be used as a helpful prognostic marker. Moreover, these parameters in combination can aid in predicting the recurrence rate in patients receiving therapy. Therefore, the present study was undertaken to study the association between Ki-67 Immuno-expression and histopathological grade along with serum PSA levels in Adenocarcinoma of Prostate.

Material and Methods

Study design and sample collection

A prospective study on 27 cases of prostate adenocarcinoma diagnosed on biopsy material as well as prostate specimens was conducted over a period of 1.5 years from January 2018 to June 2019. The ethical clearance from institutional ethical committee was obtained for the present study. All relevant clinical details including age, clinical manifestations, Digital Rectal Examination, serum Prostate Specific Antigen (PSA) levels and imaging (if any) were obtained

from electronic medical record system. All cases of prostatic adenocarcinoma were fixed in 10% formalin followed by paraffin embedding and staining with haematoxylin and eosin. Histopathological grade was assigned using Gleason's grading system (score <7 -well differentiated; score 7 to 8 - moderately differentiated; score 9 to 10 - poorly differentiated). Grading was done on the basis of recent WHO grade group (Grade group 1 - Gleason's score ≤ 6 ; Grade group 2 - score $3+4=7$; Grade group 3 - score $4+3=7$; Grade group 4 - score 8; Grade group 5 - score 9 to 10).

Immunohistochemistry using Ki-67 proliferative marker was performed on 3 μ m thick paraffin embedded sections taken on poly-L-lysine coated slides. Antigen retrieval was done by trisodium citrate buffer at a pH of 6. Monoclonal antibody of Ki-67 (Novocastra) was used for antigen detection by one step Horseradish Peroxidase (HRP) polymer method. Brown granular nuclear reactivity was considered as positive. Ki-67 labeling index was expressed as percentage of positively stained cells per 100 epithelial cells after counting at least 1000 cells using high power. Area with maximum proliferation was chosen to evaluate the labeling index. Labeling index for Ki-67 was categorized as follows: < 2% - negative; 2 to < 25% = 1+; 26 - 50% = 2+; >50% = 3+. Ki-67 index was then correlated with the histopathological grade and serum PSA levels.

Statistical Analysis

Descriptive statistical analysis was performed and the results were presented in frequencies, percentages and mean \pm SD. The Chi-square test was used to compare the categorical variables. The Mann-Whitney U test was used to compare continuous variables. The p-value of <0.05 was considered significant.

Results

A total of 27 cases of prostate adenocarcinoma were included in the present study. Amongst 27 cases, 18 (66.66%) were prostate specimens and 9 (33.33%) were biopsies. Out of these 27 cases, 13

patients (48.1%) were in the age range of 61-70 years, 7 patients (25.9 %) were below 60 years and 7 (25.9%) were in the age range of more than 70 years. The mean age of presentation was 64.9 years.

Most common presenting complaint was acute urinary retention (67%), followed by dysuria (19.4%) and nocturia (13.6%). On Digital rectal examination (DRE), 11cases (40.74%) showed hard consistency, 6 cases (22.22%) were nodular, 4 cases (14.8%) were firm in consistency, 4 cases (14.8%) showed fixity and 2 cases (7.4%) had obliteration of left lateral sulcus. Serum PSA levels was more than 20 ng/ml in 8 cases (29.6%), 16 cases revealed serum PSA levels between 4.1 to 20 ng/ml, and 3 cases (7.4%) showed serum PSA levels below 4 ng/ml.

Out of 27 cases, 2 cases were diagnosed as well differentiated adenocarcinoma (Fig 1), 16 cases were classified as moderately differentiated (Fig 2) and remaining 9 cases were reported as poorly differentiated tumors (Fig 3).

Out of 27 cases, Ki-67 expression was found to be positive in 18 cases (66.6%) while remaining 9 cases were negative. Among 18 cases, 8 cases (29.62%) showed Ki67 labeling index of 1+, 7 cases (25.9%) showed 2+ intensity and 3 cases

(11.11%) revealed Ki-67 expression of 3+. Both the cases of well differentiated adenocarcinoma were Ki-67 negative. Among 16 cases of moderately differentiated tumors, Ki-67 positivity was noted in 10 cases (62.5%) (Fig 4). Among 9 cases of poorly differentiated tumors, 8 cases (88.88%) showed strong Ki-67 positivity (Table 1) (Fig 5).

Among 8 cases (29.6%) with serum PSA levels more than 20ng/ml, Ki-67 index was positive in 6 cases (75%). Out of 16 cases (59.2 %) with serum PSA levels ranging from 4.1 to 20ng/ml, high Ki-67 index was seen in 10 cases (63%). There was no significant ($p>0.05$) association of serum PSA levels with Ki-67 among Prostate Adenocarcinoma cases.

In the present study, out of 27 cases, 25 cases (92.6%) with Gleason's score of more than or equal to 7 were observed. Out of these 25 cases, 18 cases (72.0%) showed strong Ki-67 positivity. Thus, there was significant association ($p=0.03$) of Gleason's score with Ki-67 labeling index among these tumors. Out of 27 cases, Grade group 5 was seen in 9 cases (33.3%), Grade group 4 was seen in 2 cases (7.4%), followed by Grade group 3 in 3 cases (11.1%), Grade group 2 in 11 cases (40.7%) and Grade group 1 in 2 cases (7.4%).

Table 1: Association between Ki-67 index and Gleason's Grade in prostatic adenocarcinoma

Ki-67 (Labeling Index)		Gleason's Grade					
		Well differentiated (≤ 7)		Moderately differentiated (7-8)		Poorly differentiated (9-10)	
Interpretation	No. of stained cells	Ki-67 +	Ki-67 -	Ki-67+	Ki-67 -	Ki-67+	Ki-67-
0	$\leq 2\%$	0	2	0	6	0	1
1+	3-25%	0	0	5	0	3	0
2+	26-50%	0	0	4	0	3	0
3+	$>50\%$	0	0	1	0	2	0
Total		0	2	10	6	8	1

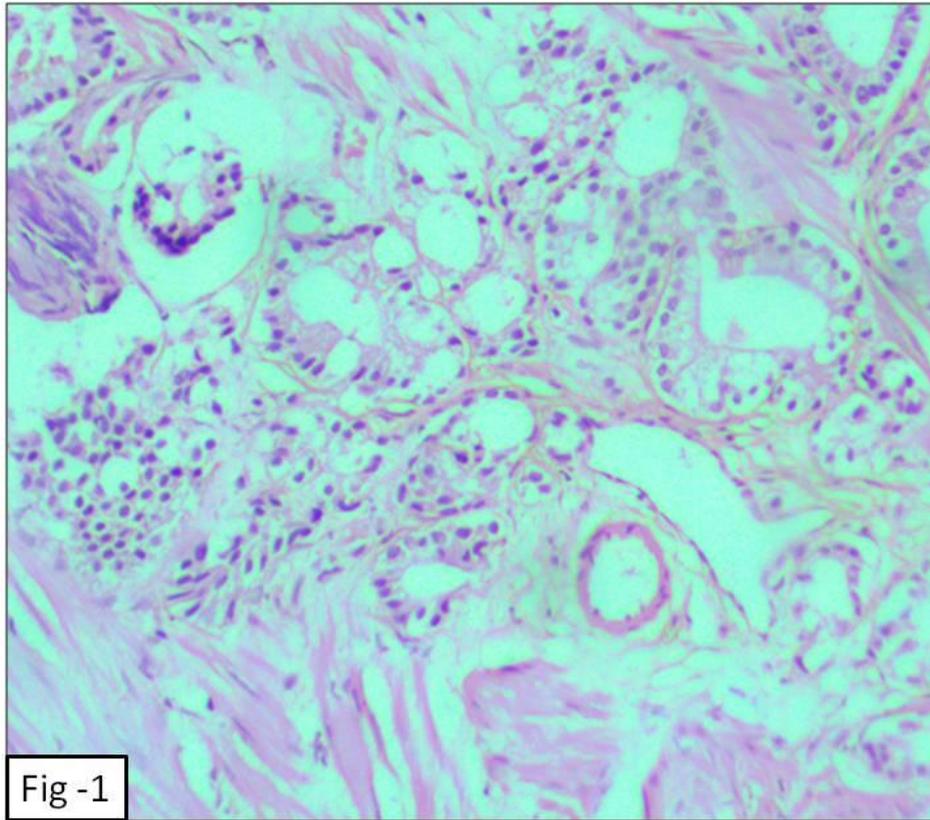


Figure-1: Photomicrograph showing well differentiated prostatic adenocarcinoma (H&E, 10X),

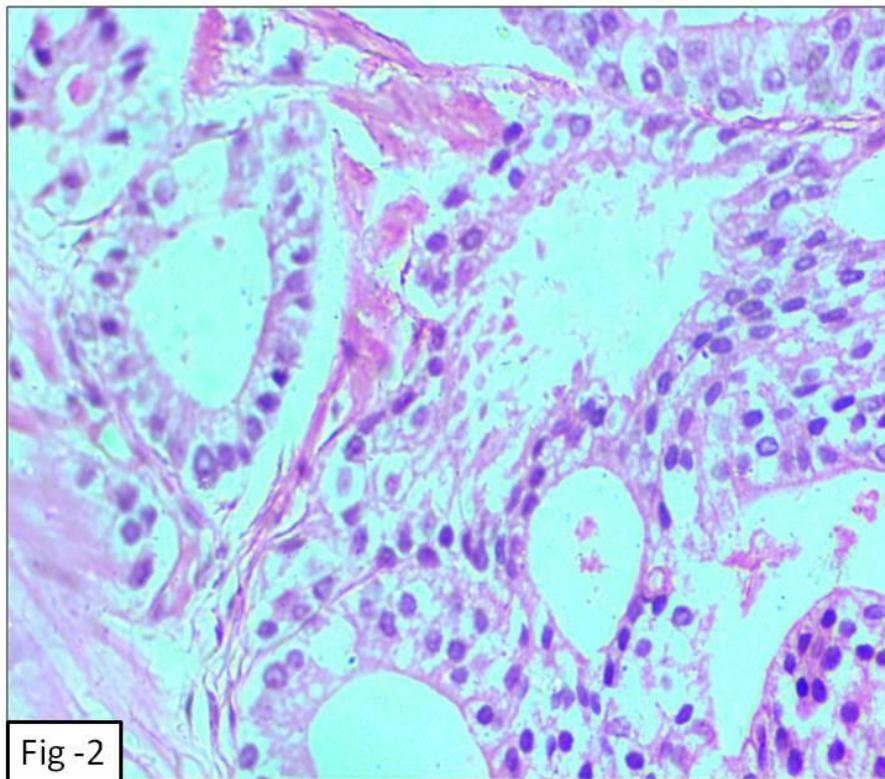


Figure-2: Photomicrograph showing moderately differentiated prostatic adenocarcinoma (H&E, 40X),

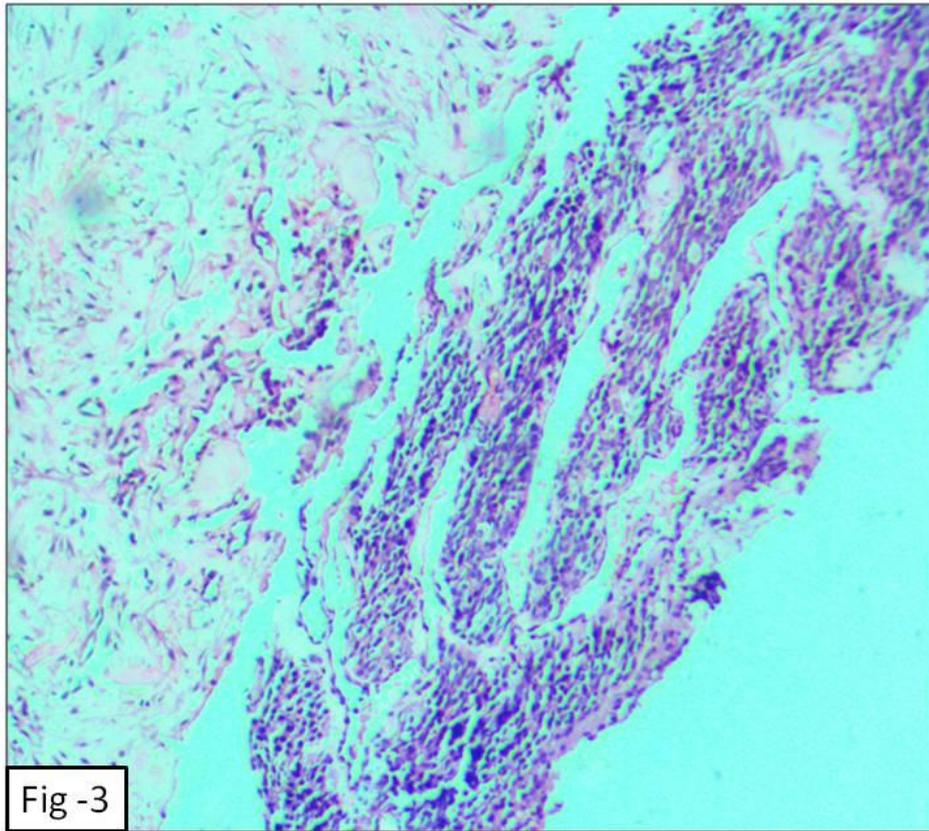


Figure-3: Photomicrograph showing poorly differentiated prostatic adenocarcinoma (H&E, 10X)

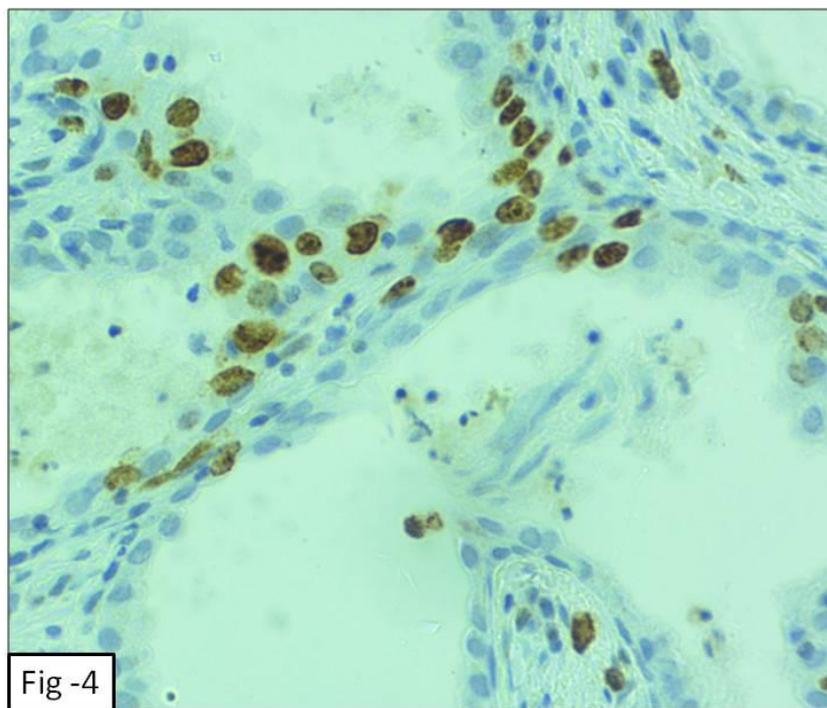


Figure-4: Photomicrograph showing strong Ki-67 positivity in moderately differentiated tumour (IHC, 40X)

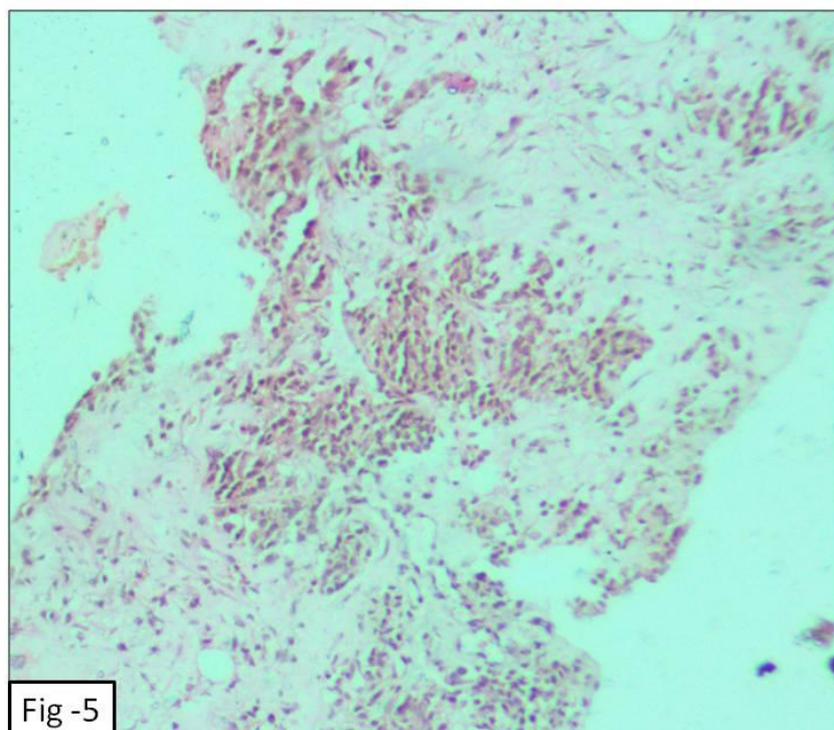


Figure-5: Photomicrograph showing strong Ki-67 positivity in poorly differentiated tumour (IHC, 10X)

Discussion

In the present study, out of 27 cases majority of the patients were in the age range of 61-70 years with mean age of presentation being 64.9 years. In a study by Hirachand et al., maximum number of cases (n=47; 36.72%) were in the age group of 61-70 years followed by 71-80 years age group (n=43; 33.5%).³ Similar findings were reported by Lakhey et al (2010)⁴, Josephine (2014)⁵ and Lokuhetty et al., (2009)⁶ and Mansoor et al., (2003)⁷ with mean age of presentation being 65.5, 65.55, 67.61, 69.7 years respectively.

Of the 27 cases, majority of the cases were prostatectomy specimens (66.66%) while remaining were needle biopsies (33.33%). This was similar to the findings by Josephine et al.(2014)⁵, with 42% needle biopsies and 58% of the resection specimens. The most common presentation among these patients was acute urinary retention (67%), followed by dysuria (19.4%) whereas in a stud by Josephine et al., dysuria was the most common (81% cases) presentation.⁵

In the past, digital rectal examination was considered as a screening tool for the detection of prostate cancers but currently emphasis has been shifted to measurement of PSA levels. However PSA test is not a specific test and confirmation of prostate cancer is carried out only through histopathological analysis of biopsy sample. In the present study, majority of the cases showed hard consistency on digital rectal examination and this was in concordance with the findings of Edoise et al.,⁸ Out of 30 cases, 24 cases revealed serum PSA levels above 4ng/ml, of these cases only 19 of the cases showed serum PSA levels above 10ng/ml. In a study by Murti et al., out of 30 samples of prostate adenocarcinoma 21 cases revealed serum PSA levels above 10ng/ml as was observed in the present study.⁹ Therefore, no significant ($p>0.05$) association of serum PSA levels with Ki-67 among Prostate Adenocarcinoma (PA) cases was noted as observed by other authors like Sulik M et al. and Berney et al.^{10,2}

In the present study, 10 of 16 cases (62.5%) of moderately differentiated type and 8 of 9 cases (88.88%) of poorly differentiated

adenocarcinomas revealed Ki-67 positivity. In a study by Madani et al., Ki-67 was negative in well differentiated tumors but was strongly positive in 13 of 21 cases (61.90%) of moderately differentiated tumors and 22 of 25 cases (88%) of poorly differentiated tumors.¹¹

In the present study, Ki-67 expression was found to be positive in 18 of 27 cases (66.6%). This finding was in concordance with the findings of Nikoleisvili et al.,¹² Out of 18 cases, 2+ intensity was observed in 7 cases (26%) while 3+ intensity was seen in 3 cases (11%). Of these 18 cases, 10 cases were moderately differentiated while 8 cases were poorly differentiated. Among 10 cases of moderately differentiated tumors, 1+ positivity was seen in 5 cases followed by 2+ in 4 cases and followed by 3+ in 1 case. Among 8 cases of poorly differentiated tumors, Ki-67 with 1+ positivity was seen in 3 cases, followed by 2+ in 3 cases and 3+ in 2 cases. Similar findings were observed by Verma et al., with none of the well differentiated tumors showing any Ki-67 reactivity.¹³ Thus, Ki-67 expression was significantly up regulated in prostate cancers and highest proliferative indices was noted in poorly differentiated tumors indicating that Ki-67 expression is increased in aggressive and high grade prostatic carcinomas.

In the present study there was significant ($p=0.03$) association of Gleason's score with Ki-67 index among prostatic adenocarcinoma cases which is in concordance with the findings of Verma et al., ($p=0.002$) and Fenley et al., ($p=0.02$).^{13,14} Thus, exhibiting the fact that Ki-67 expression helps in identification of tumors with high rate of cell growth and, thereby, is a good prognostic marker.

Conclusion

It is concluded that frequency of expression of Ki-67 is significantly up regulated in prostatic carcinomas and increased expression is usually noted in aggressive and high grade prostatic tumors. In the present study, Ki-67 expression was markedly high in tumors with increased Gleason's grade which reflects its crucial relationship with

the prognosis of these cancers. Further intensive studies including long term follow up are needed in the future to understand the biological behaviour of these cancers and their association with immunomarkers that are of prognostic significance.

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Conflict of interest: None

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