ORIGINAL RESEARCH PAPER

Pathology

ASSESSMENT OF KI-67 EXPRESSION IN CASES OF PROSTATIC CARCINOMA AND ITS CORRELATION WITH HISTOPATHOLOGICAL PROGNOSTIC PARAMETERS AND PROSTATE-SPECIFIC ANTIGEN [PSA]

KEY WORDS: Ki-67, Prostatic cancer, Gleason's grade, Prostate-Specific Antigen

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ABSTRACT

Background: The high prevalence of low-grade prostate cancer and its long natural history, competing causes of death in older men and treatment patterns of prostate cancer, have led to dramatic overtreatment of the disease. Improved markers of prostate cancer lethality are needed to reduce the overtreatment of prostate cancer. In the current study we evaluated the expression of Ki-67 in cases of prostatic carcinoma and correlated its expression with histopathological prognostic parameters and Prostate-Specific Antigen (PSA) level.

Methods: It was a single centre descriptive type of study. Total 50 cases were included. Patients who visited the urology department and whose biopsies were received in the department of pathology from 2015 to 2018 for routine reporting and diagnosed to have adenocarcinoma were included. Brief clinical data was noted from case records including age, presenting symptoms, serum PSA levels, radiological findings and clinical diagnosis. Histopathological prognostic parameters were recorded on routine haematoxylin and eosin (H&E) stain and Ki-67 expression was recorded on Immunohistochemical stain.

Results: In the current study, 78% cases of carcinoma prostate were positive for Ki-67 expression. There was significant positive correlation of Ki-67 expression with Gleason's grade and Modified Gleason's grading system, however there was no significant correlation of Ki-67 expression with PSA level among the cases of prostate carcinoma.

Conclusion: Ki-67 can be used along with the other established prognostic parameters to assess the lethality of prostate cancer.

INTRODUCTION

Prostatic carcinoma is increasing frequently with advancing age. It is an important growing health problem with often unpredictable clinical course. Specific molecular mechanisms are involved in the development and progression of prostatic cancer. Therefore, much research has been dedicated in identifying prognostic factors that distinguish indolent versus aggressive forms of prostate cancer.

Tumor differentiation and proliferative activity are important predictors of biological behavior. While routine histopathological evaluation is fairly adequate to assess differentiation, tumor proliferative activity is difficult to measure. Ki-67 staining index is reported to be useful for assessing tumor proliferation. The Ki-67 is a protein that is expressed in G1, G2, S and M phases of the cell cycle. The presence of this protein can be demonstrated by immunohistochemistry assays.[1]

In the present study we assessed the immunohistochemical expression of Ki-67 in cases of prostatic carcinoma and correlated its expression with histopathological prognostic parameters and PSA.

METHODS

It was a single centre descriptive type of study. Total 50 cases were included.

Study Population:-

Both IPD and OPD patients who visited the urology department and whose biopsies were received in the department of pathology (TURP chips/ Tru cut Prostatic biopsies as well as radical prostatectomies) from 2015 to 2018 for routine reporting. All specimens found positive for adenocarcinoma were included.

Inclusion Criteria:-

1) Cases of Adenocarcinoma

Exclusion Criteria:-

1) Other than Adenocarcinoma
2) Inadequate biopsies
3) Poorly preserved prostatic specimen
4) Marked inflammation
5) Pateints which were lost on follow up

Brief clinical data was noted from case records, which included age, presenting symptoms, serum PSA levels, radiological findings and clinical diagnosis.

Histopathological prognostic parameters were recorded on routine haematoxylin and eosin (H&E) stain. H&E staining was done as per the standard procedure.[2]

Morphological Evaluation

Prostate fragments were fixed in 10% formalin, paraffin-embedded, sectioned and standard H and E stained sections were studied under light microscope. Carcinoma cases were histologically graded according to Gleason's grading system[3] and Modified Gleason's grading system [ISUP grade].[4]

Immunohistochemical Analysis

Ki-67 expression was recorded on Immunohistochemical stain. IHC staining was done as per the standard procedure.[5]

Ki-67 Expression:

Dark brown nuclear staining was taken as positive.

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. In this study, at least 500 cells were counted and only the cells that were definitely positive for the desired marker were considered. Based on Ki-67 Staining index the tumors were divided into five groups.[6]

Negative = when percentage of stained cells was less than 2%
1+ = when 2 to 25% cells were stained

2+ = when 26-50% cells were stained
3+ = when 51-75% cells were stained
4+ = when 76-100% cells were stained.

Statistical Analysis:
All statistical analyses were performed using the statistical package IBM SPSS, version 24. Results were presented as tables and charts. Continuous variables were presented as mean ± SD and categorical variables as percentages. Spearman's correlation coefficient was used to assess the correlation between Ki-67 expression and Histopathological prognostic parameters and PSA. Probability less than 0.05 was considered as significant.

ETHICAL CONSIDERATION
The study protocol was approved by the institutional ethics committee.

RESULTS AND OBSERVATIONS
Table 1: Characteristics Of Research Samples

Characteristics	Total	Percentage
Age		
51-60	2	4
61-70	13	26
71-80	26	52
81-90	9	18
Nature Of Surgical Specimen		
Needle Core Biopsy	31	62
TURP	9	18
Prostatectomy Specimen	10	20
PSA LEVEL		
<4	1	2
4-10	2	4
11-20	2	4
21-30	4	8
31-40	3	6
41-50	8	16
>50	30	60
GLEASON'S SCORE		
Well differentiated <7	5	10
Moderately differentiated =7	33	66
Poorly differentiated >7	12	24
MODIFIED GLEASON'S GRADE (ISUP GRADE)		
GRADE 1 (3+3)	5	10
GRADE 2 (3+4)	11	22
GRADE 3 (4+3)	22	44
GRADE 4 (4+4)	4	8
GRADE 5 (>8)	8	16'
Ki-67 EXPRESSION		
Negative (<2%)	11	22
1+ (2-25%)	22	44
2+ (26-50%)	9	18
3+ (51-75%)	6	12
4+ (76-100%)	2	4

Table: 1 illustrates Characteristics of Research Samples

Mean age amongst the cases was 72.8±7.35 (years). The youngest patient was 52 years old and oldest patient was 88 years old. It was observed that maximum numbers of cases were in the age group of 61-80 years forming 78% of the total cases.

There were 31 (62%) cases of needle core biopsy, 9 (18%) cases of transurethral resection of prostate (TURP), while 10 (20%) prostatectomy specimen was received. So, Needle core biopsy was the most common surgical procedure performed for the histopathological diagnosis of carcinoma prostate.

Maximum number of cases i.e. 30 (60%) out of 50 had a serum PSA level >50 ng/ml while only one case (2%) had normal level (<4ng/ml).

Among 50 cases, maximum i.e. 33 (66%) were moderately differentiated with a score of 7 while 12 cases (24%) were poorly differentiated with a score of >7 and only 5 cases (10%) were well differentiated with a score of <7.

Among 50 cases, maximum i.e. 22 (44%) cases had group grade 3 pattern, 11 (22%) cases had group grade 2 pattern, 8 (16%) cases had group grade 5 pattern, 5 (10%) cases had group grade 1 pattern and 4 (8%) cases had group grade 4 pattern.

11 of 50 (22%) cases of carcinoma prostate were negative (index<2%) for Ki-67 while 39 (78%) cases were positive for Ki-67. Among 39 (78%) positive cases maximum number of cases 22 (44%) showed 1+ positivity followed by 9 (18%) cases with 2+ positivity, 6 (12%) cases with 3+ positivity and 2 (4%) cases with 4+ positivity.

Table 2: Frequency Of The Ki-67 Staining Index In Relation To Tumor Differentiation And Gleason's Score In Cases Of Carcinoma Prostate

Ki-67 Staining index	GLEASON'S SCORE			Total
	Well differentiated tumors (%) (<7)	Moderately differentiated tumors (%) (=7)	Poorly differentiated tumors (%) (>7)	
Negative (<2%)	4 (80%)	7 (21.21%)	0	11
1+ (2-25%)	1(20%)	20 (60.60%)	1 (8.33%)	22
2+ (26-50%)	0	6 (18.18%)	3 (25%)	9
3+ (51-75%)	0	0	6 (50%)	6
4+ (76-100%)	0	0	2(16.66%)	2
Total	5 (100%)	33 (100%)	12 (100%)	50

Table: 2 illustrates frequency of Ki-67 staining index in relation to tumor differentiation and Gleason's score of prostatic carcinoma.

Out of 5 cases of well differentiated tumors, maximum i.e. 4 (80%) cases were negative for Ki-67 expression and only one (20%) case showed 1+ positivity.

Out of 33 cases of moderately differentiated tumors, maximum cases i.e. 26 (78.78%) were positive for Ki-67 expression, including 20 (60.60%) cases with 1+ positivity and 6 (18.18%) cases with 2+ positivity while only 7 (21.21%) cases were negative for Ki-67 expression.

All 12 (100%) cases of poorly differentiated tumors were positive for Ki-67 expression, including 1 (8.33%) case with 1+ positivity, 3 (25%) cases with 2 + positivity, 6 (50%) cases with 3+ positivity and 2 (16.66%) cases with 4 + positivity.

Consequently a statistically significant positive correlation was observed between Ki-67 positivity and Gleason's score of prostatic carcinoma as indicated by **correlation coefficient r=0.758, p value of <0.001**.

Table 3: Frequency Of The Ki-67 Staining Index In Relation To Modified Gleason's Grade (ISUP Grade)

Ki-67 Staining index	MODIFIED GLEASON'S GRADE (ISUP GRADE)					Total
	GROUP GRADE 1	GROUP GRADE 2	GROUP GRADE 3	GROUP GRADE 4	GROUP GRADE 5	
Negative (<2%)	4 (80%)	4 (36.36%)	3 (13.63%)	0	0	11

1+ (2-25%)	1(20%)	7 (63.63%)	13 (59.0%)	1(25%)	0	22
2+ (26-50%)	0	0	6 (27.27%)	1(25%)	2(25%)	9
3+ (51-75%)	0	0	0	2(50%)	4(50%)	6
4+ (76-100%)	0	0	0	0	2(25%)	2
Total	5 (100%)	11 (100%)	22 (100%)	4 (100%)	8 (100%)	50

Table: 3 illustrates frequency of Ki-67 labeling index in relation to Modified Gleason's Grade of prostatic carcinoma. Out of 5 cases of Group Grade 1 tumors, maximum i.e. 4 (80%) cases were negative for Ki-67 expression and only one (20%) case showed 1+ positivity.

Out of 11 cases of Group Grade 2 tumors, maximum i.e. 7 (63.63%) cases showed 1+ positivity and 4 (36.36%) were negative for Ki-67.

Out of 22 cases of Group Grade 3 tumors, maximum cases i.e. 19 (86.27%) were positive for Ki-67 expression, including 13 (59%) cases with 1+ positivity and 6 (27.27%) cases with 2+ positivity while only 3 (13.63%) cases were negative for Ki-67 expression.

All 4 (100%) cases of Group Grade 4 tumors were positive for Ki-67 expression, including 1 (25%) case with 1+ positivity, 1 (25%) case with 2 + positivity, 2 (50%) cases with 3 + positivity.

All 8 (100%) cases of Group Grade 5 tumors were positive for Ki-67 expression, including 2 (25%) cases with 2+ positivity, 4 (50%) case with 3 + positivity and 2 (25%) cases with 4 + positivity.

Consequently a statistically significant positive correlation was observed between Ki-67 positivity and Modified Gleason's grade of prostatic carcinoma, indicated **by correlation coefficient r=0.799, p value of <0.001**.

Table 4: Correlation Of Ki-67 Expression With PSA Levels In Cases Of Prostatic Carcinoma

PSA LEVEL	Ki-67 Staining index					Total
	Negative (<2%)	1+ (2-25%)	2+ (26-50%)	3+ (51-75%)	4+ (76-100%)	
<4	0	1 (4.5%)	0	0	0	1
4-10	1 (9.09%)	0	1 (11.11%)	0	0	2
11-20	1 (9.09%)	1 (4.5%)	0	0	0	2
21-30	1 (9.09%)	1 (4.5%)	2 (22.22%)	0	0	4
31-40	1(9.09%)	2 (9.0%)	0	0	0	3
41-50	2 (18.18%)	3 (13.63%)	2 (22.22%)	0	1 (50%)	8
>50	5 (45.45%)	14 (63.63%)	4 (44.44%)	6 (100%)	1 (50%)	30
Total	11 (100%)	22 (100%)	9 (100%)	6 (100%)	2 (100%)	50

Table: 4 illustrates correlation of Ki-67expression with PSA Levels in cases of Prostatic Carcinoma.

One case in which PSA Level was <4 showed 1+ positivity for Ki-67.

Out of 2 cases in which PSA Level was 4-10, one case was negative for Ki-67 and one case showed 2+ positivity 2 case in which PSA Level was 11-20, one case was negative for Ki-67 and one case showed 1+ positivity

4 cases in which PSA Level was 21-30, one case was negative for Ki-67, one case showed 1+ positivity and 2 cases showed 2+ positivity

3 cases in which PSA Level was 31-40, one case was negative for Ki-67 and 2 cases showed 1+ positivity

8 cases in which PSA Level was 51-50, 2 cases were negative for Ki-67, 3 cases showed 1+ positivity, 2 cases showed 2+ positivity and 1 case showed 4+ positivity

Among 30 cases in which PSA Level was >50, 5 case were negative for Ki-67, 14 cases showed 1+ positivity, 4 cases showed 2+ positivity, 6 cases showed 3+ positivity and 1 case showed 4+ positivity

There was no statistically significant correlation between Ki-67 expression and PSA Levels among the cases of Prostatic Carcinoma as indicated **by correlation coefficient r=0.208, p value of 0.148**.

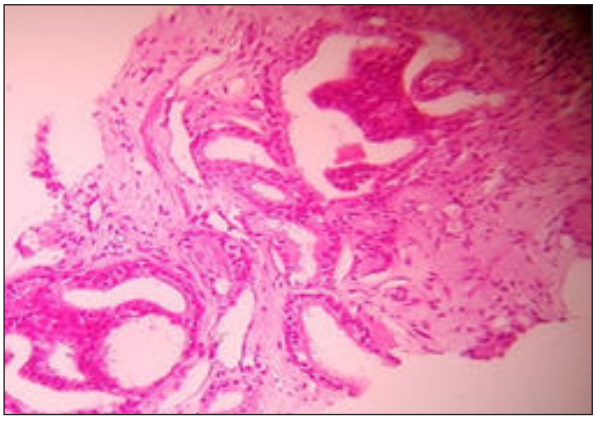

Figure 1: Well differentiated prostatic carcinoma (40x)

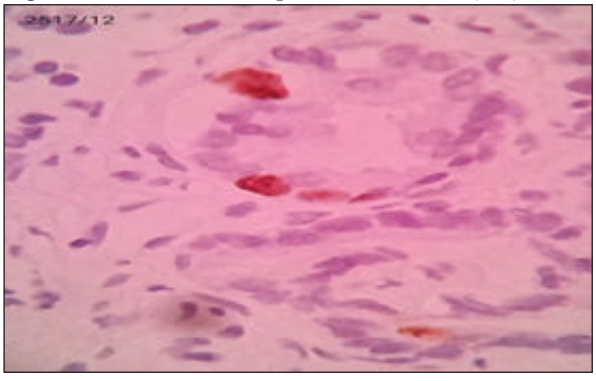

Figure 2. Well differentiated prostatic carcinoma showing 5% Ki 67 positivity (40x)

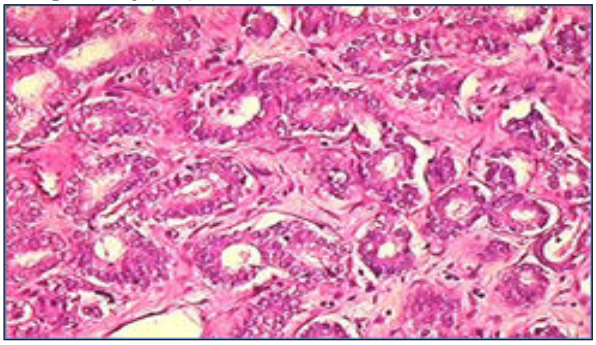

Figure 3: Moderately differentiated prostatic carcinoma (40x)

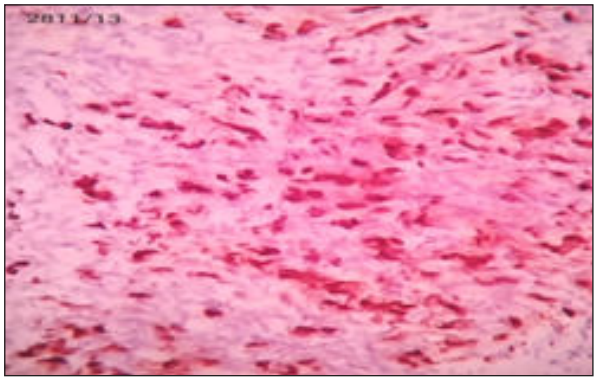

Figure 4: Moderately differentiated prostatic carcinoma showing 46% Ki-67 positivity (40x)

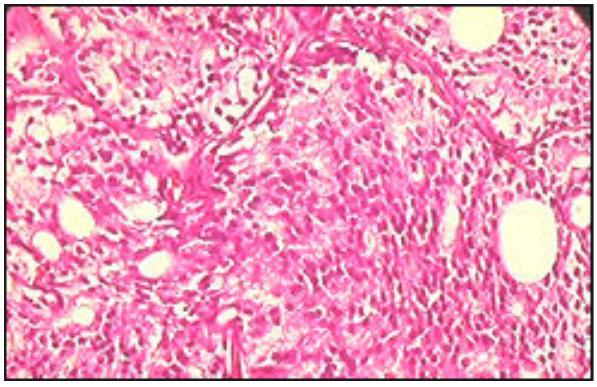

Figure 5: Poorly Differentiated Prostatic Carcinoma (40x)

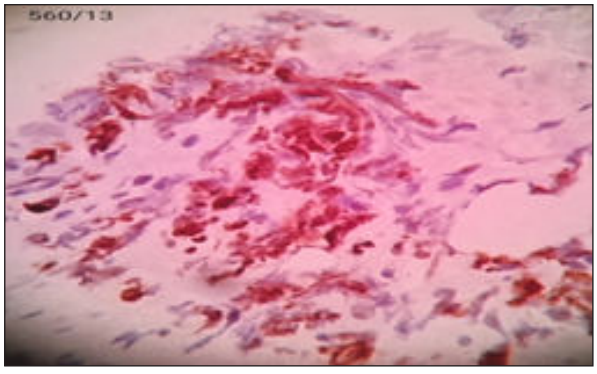

Figure 6. Poorly differentiated prostatic carcinoma showing 90% Ki-67 positivity (40x)

DISCUSSION

Ki-67 is a protein situated inside the cells and is linked with cellular proliferation. It is expressed in all the phases of cell cycle but can be detected only in malignant cells while it goes undetected in resting cells. The present study was planned to assess the expression of Ki-67 in prostatic carcinoma and to correlate its expression with clinicopathological prognostic parameters.

Total of 50 cases of prostatic carcinoma were studied. 4-5µm thick sections were prepared and stained routinely with hematoxylin and eosin (H&E) and were graded histologically based on Gleason's and modified Gleason's grading system. A section from each case was subjected to immunohistochemical (IHC) staining for Ki-67. The data of observation presented in our study is discussed as under.

In the present study, among 50 cases of carcinoma prostate, Ki-67 was expressed in 39 (78%) cases. Mean Ki-67 was 15.23%, ranging from 0 to 76.1%. Similarly in the study done by **Samia M. Gabal et al** 80% cases of carcinoma prostate showed Ki-67 expression.[7] In the study done by **Renuka Verma et al** 64% cases of carcinoma prostate showed Ki-67 expression.[8] In the study by **Paz JI et al** all cases of carcinoma prostate were positive for Ki-67 expression.[9]

Correlation Of Ki-67 Expression In Relation To Histologic Grade Based On Gleason's Score In Cases Of Carcinoma Prostate

In our study, 4 of 5 (80%) cases of well differentiated tumors were negative for Ki-67 expression and only one (20%) case was positive. 26 of 33 (78.78%) moderately differentiated and all 12 (100%) cases of poorly differentiated tumors were positive for Ki-67 expression. So there was statistically significant positive correlation between Ki-67 expression and histologic grade based on Gleason's score ($p<0.05$). This was in concordance with the study done by Madani et al in which Ki-67 was negative in all 3 (100%) well differentiated tumors. 13 of 21 (61.90%) moderately differentiated tumors and 22 of 25 (88%) poorly differentiated tumors were positive for Ki-67.[6] Similarly other studies have also shown positive correlation between Ki-67 expression and Gleason's score.[7,10,11,12,13,14,15,19] These observations indicate that greatest proliferative indices are noted in poorly differentiated tumors concluding that Ki-67 index increases in aggressive and high grade prostatic carcinoma. Thus Ki-67 can be used as an independent prognostic marker in cases of prostate cancer.

Correlation Of Ki-67 Expression In Relation To Modified Gleason's Grade

Similarly the present study revealed a statistically significant positive correlation of Ki-67 expression with Modified Gleason's grade ($p<0.05$). This was similar to the previous studies who found significant positive correlation between Ki-67 expression and Modified Gleason's grade in cases of prostatic carcinoma.[10,16]

Table: 5 Correlation Of Ki-67 Expression With Gleason's Grade In Cases Of Prostatic Carcinoma In Different Studies

S. No	Study	Year	No. of cases	Correlation of ki-67 positivity with histological grade
1.	Madani et al[6]	2011	49	Significant
2.	Krisna Murti et al[10]	2017	30	Significant
3.	Samia M. Gabal et al[7]	2017	25	Significant
4.	Harjot Kaur et al[11]	2016	50	not significant
5.	Rajeswari K et al[12]	2016	46	Significant
6.	Shane Mesko et al[13]	2013	77	Significant
7.	Lukas Bubendorf et al[14]	1998	111	Significant
8	Marie-Claude Bettencourt et al[15]	1996	180	Significant
9.	Elin Richardsen[16]	2017	535	Significant
10.	Didier Cowen et al[17]	2002	106	Significant
11.	Aaltomaa et al[18]	1997	190	Significant
12.	Marian Sulik et al[19]	2011	56	Significant
13.	Renuka Verma et al[8]	2015	50	Significant
14.	Present study	2020	50	Significant

Correlation Of Ki-67 Expression With PSA Level

There was no significant correlation of Ki-67 expression with PSA Levels among the cases of Prostatic Carcinoma in the present study. This was in concordance with the previous studies who found no significant correlation between Ki-67 expression and serum PSA levels in cases of carcinoma prostate.[7,10,19,20] This can be explained by the fact that Serum PSA levels correlate with the extent of cancer (stage) and histological grade. However, volume for volume, cancer produces less PSA than BPH, and poorly differentiated prostate cancers produce less PSA than well-differentiated cancers.[21] But, volume for volume, more PSA leaks into the circulation due to prostate cancer than BPH,[22] The variable

contribution to PSA from benign tissue and the non-linear relationship between grade and PSA lead to overlap in PSA levels between stages.[21] As a result, PSA cannot be used alone to accurately predict disease extent for any individual patient.

CONCLUSIONS

Ki-67 expression, a cell proliferation marker is significantly up-regulated in malignant lesions. As most cases of prostate cancer are diagnosed microscopically before metastatic spread and among these only few cases have poor outcome. So our aim is to differentiate indolent versus aggressive forms of prostate cancer in order to avoid overtreatment in patients who otherwise would benefit from conservative treatment. In the current study, Ki-67 marker was shown to have a strong correlation with increased Gleason's grade and modified Gleason's grade which has a strong relationship with the prognosis of prostate cancer. Therefore, we propose that this marker can be used along with other established prognostic factors to assess the aggressiveness of prostate cancer. However, further large prospective clinical studies along with long-term follow-up are needed to assess the prognostic significance of Ki-67 in patients with prostate carcinoma.

Source Of Funding

The entire study was done using available resources at our institute and no external funds were sought / utilized.

Conflict Of Interest- Nil

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