Original Article

Ki-67 and p53 Immunohistochemical Expression in Prostate Carcinoma: An Experience from a Tertiary Care Centre of North India

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ABSTRACT

Background: Carcinoma of the prostate poses a considerable medical and public health challenge in many parts of the world. Despite advances in screening and multimodal management of this disease, overall survival remains poor. The need to identify tumor markers as prognostic indicators and as targets for new therapeutic strategies, still remains a major challenge in prostate carcinoma research.

Material: The study was conducted on 50 histopathologically proven cases of prostate carcinoma received in the Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, Punjab, India. The aim was to evaluate the immunohistochemical expression of Ki-67 antigen and p53 protein in prostate carcinoma and to find their correlation with other clinicopathological parameters. The results obtained were tabulated and statistically analyzed. A p value less than 0.05 was considered statistically significant.

Results: Maximum cases (74%) were observed in the age group of 61 – 80 years. The most common pattern of Gleason grade seen was (5+4) constituting 32% of the total cases. The most common Gleason score seen was 7 - 8 constituting 48% of the total cases. The Ki-67 positivity was observed in 80% of cases with percentage positive cells varying from 3-84% with moderate and strong staining intensity. It was observed that with increase in the grade and score the number of cases showing positivity also increased but no statistical significance was seen with the same.

The p53 positivity was observed in 58% of cases with percentage positive cells varying from 5-80% with mild, moderate and strong staining intensity. It was observed that with increase in the grade and score the number of cases showing positivity also increased but no statistical significance was seen with the same. 24 cases (48.0%) were positive for both Ki-67 and p53. Of the 24 cases of intermediate grade tumors positivity for both Ki-67and p53 was noted in 11 cases (45.8%) but no statistical significance was observed with increase in grade and score.

Conclusion: The present study highlights that the immunohistochemical expression of Ki-67 and p53 should be assessed in all the cases of prostate carcinoma as these markers allow identification of tumors with a higher rate of cell growth. They also permit development of prognostic factors as their expression increases with increase in the grade and these patients can be benefited with the appropriate targeted treatment leading to increase in the survival time.

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Introduction

Prostate carcinoma is a global health problem and the second leading cause of cancer related deaths in the western world with the consensus reaching to nearly the same level in the Indian subcontinent. Its incidence is continuously rising with over 200000 new cancer cases and 35000-40000 deaths per year.[1] Despite advances in screening and multimodal management of this disease, overall survival remains poor. Hence, there is a need to identify various prognostic markers for developing new therapeutic strategies for better management of prostate carcinoma patients.

There are various benign mimickers of prostate carcinoma such as benign hyperplasia, prostatitis, atrophy, adenosis, atypical adenomatous hyperplasia (AAH) and nephrogenic adenoma, which makes the diagnosis of prostate carcinoma challenging.[2]Therefore, many pathological parameters are required for its proper assessment. Among them, Gleason grading of the cancer is the most widely used, and accepted histopathological method for providing information about the prognosis of prostate carcinoma. This grading system is based entirely on the histological pattern of differentiation and arrangement of carcinoma cells and cell groups in Hematoxylin and Eosin (H&E) stained sections. [3] Other being , Prostate-specific antigen (PSA),which is the most useful tumor marker in the diagnosis of prostate carcinoma[4] but suffers from many fallacies such as borderline levels and also supuriously high levels in many reactive/ inflammatory conditions. Therefore, IHC plays a major role besides PSA and the important tumor markers are p53, Ki-67 and Bcl-2 to predict the prognosis and to decide the treatment modalities.

p53 is a tumor suppressor gene, mutations of which can result in uninhibited cellular growth and have been implicated in numerous malignancies.[5] In most human cancers, its increased immunohistochemical expression is associated with point mutations in one allele of p53 gene and loss in the other. Thomas et al and Shurbaji et al evaluated the immunohistochemical detection of p53 protein in prostate cancer and its utility as a prognostic indicator. They concluded that mutations of p53 gene, which have long half-life, are involved in carcinogenesis of prostate cancer, and that p53 reactivity marks an aggressive subset of prostate cancer.[6,7]

Ki-67 is one of several cell-cycle-regulating proteins, which can be demonstrated by IHC.[8,9] It is a DNA-binding protein, which is expressed in all phases of cell cycle but undetectable in resting cells.[10,11] McLoughlin et al studied that Ki-67 index (fraction of Ki-67 positive nuclei in IHC) was higher for carcinomas than for hyperplastic glands while within the group of carcinomas, Ki-67 indices in patients with metastatic disease were significantly higher than in those without metastasis and that high Ki-67 index could define a group of patients with poor prognosis.[10]

Borce et al showed that the accumulation of p53 had a special correlation with patient's survival in their studied population. The presence and activity of p53 was greatly associated with the cell proliferation marker Ki-67 and the level of p53 activity was an important independent prognostic factor that was inversely associated with patient survival.[12]

The present study was conducted at a tertiary care teaching hospital at Punjab (North India) to assess the expression of Ki-67 and p53 in histologically proven cases of carcinoma prostate and to evaluate any correlation between the two. An attempt was also made to correlate the expression of both the markers with other histopathological parameters such as grading and scoring.

Material and Methods

50 cases of carcinoma prostate diagnosed in needle biopsies and prostatic chips at the Department of Pathology, Sri Guru Ram Das Institute Of Medical Sciences And Research, Amritsar, Punjab, India were included in the present study. Detailed clinical data of the patient was recorded. Routine histopathological processing was done, followed by staining with H & E. The slides were observed under the light microscope, and Gleason scoring was done using the 2005 ISUP (International Society of Urological Pathology) Modified Gleason Scoring System.

IHC was done on formalin fixed and paraffin embedded 4 micron meter sections of representative blocks of each tumor and were mounted on poly-lysine coated slides. Antigen retrieval was done in a pressure cooker using sodium citrate buffer solution at pH 6.0. Peroxidase inhibition was then done, followed by washing in tris buffer saline, and protein block. To evaluate for p53 expression, the primary antibody used was CME298BK and for Ki-67 the primary antibody used was CRM325B; both procured from Biocare Medical, India. Positive and negative controls were run with every batch.

For both p53 and Ki-67: Brown nuclei were taken as positive [Figure 1and 2]. Following scoring pattern was utilized.

SCORING of p53

Percentage positivity	Score	Staining intensity	Score
<5 %	0	Nil	0
5–25 %	1	Mild	1
25–50 %	2	Moderate	2
>50 %	3	Severe/Strong	3

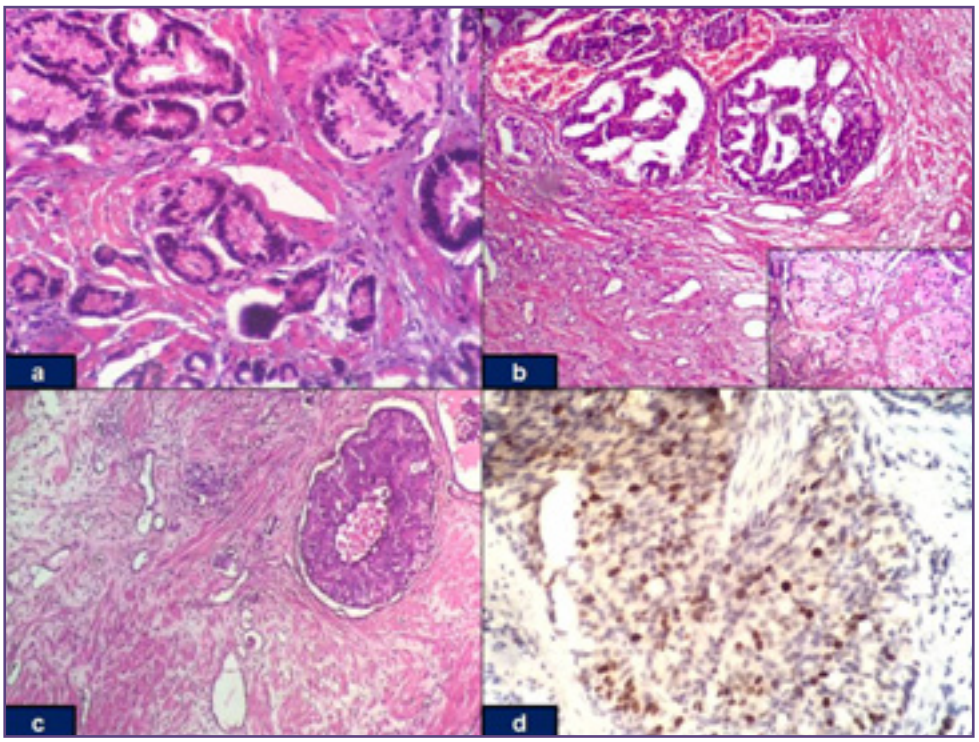

Fig. 1: a) Closely packed and irregularly separated glands (Pattern 3a) [H&E;200X]; b) Raggedly outlined fused glands (Pattern 4a)[H&E;100X] , Inset : Hypernephroid pattern with large pale cells (Pattern 4b)[H&E;400X]; c) Comedocarcinoma (Pattern 5a) [H&E;100X]; d) Tumor cells showing strong intensity (3+) p-53 expression [IHC; 400X].

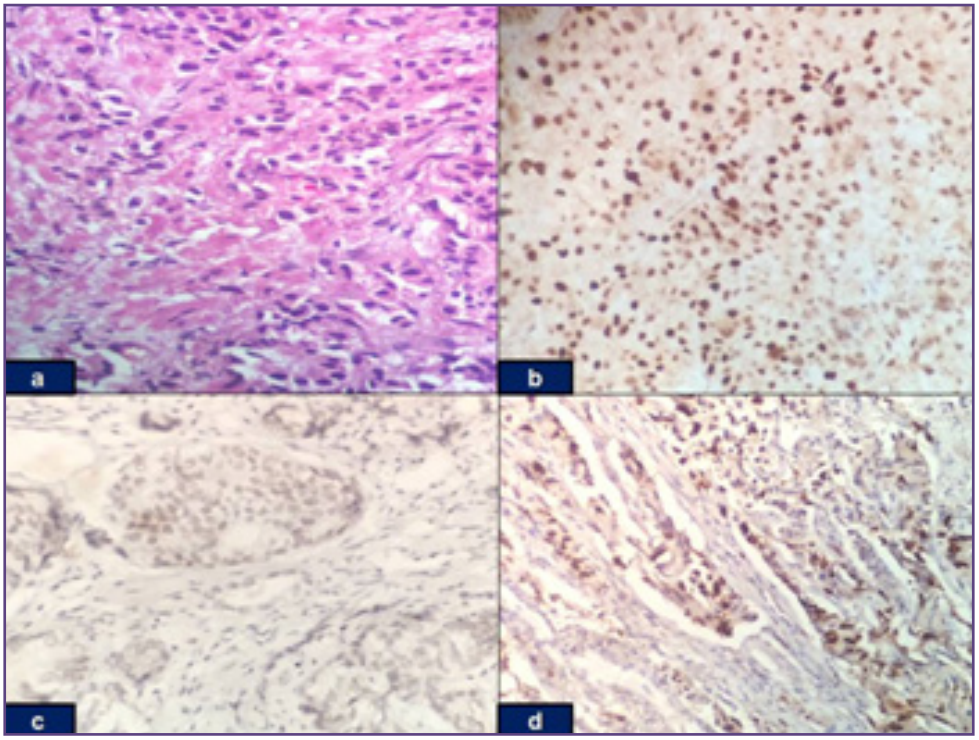

Fig. 2 : a) Diffuse growth pattern [H&E;400X]; b) Tumor cells showing moderate intensity Ki-67 expression [IHC; 200X]; c) Mild intensity nuclear expression of Ki-67 by the tumor cells [IHC; 200X]; d) Strong intensity Ki-67 immunoexpression [IHC; 200X].

Index for Ki-67

+1=Index less than equal to 25%

+2=Index between 26-50%

+3=Index between 51-75%

+4=Index between 76-100%

Statistical Analysis: The relationship between IHC expression and clinicopathological parameters was compared using Chi Square test/ Fisher's exact test wherever applicable. A value of p <0.05 was considered statistically significant.

Results

The majority of the patients were in the age group of 61-80 years constituting 74% of the total. The youngest patient was 55 years old and the eldest was 88 years. All the cases were adenocarcinoma with Gleason score varying between 6 and 10.The majority of patients had Gleason score of 7-8 constituting 48% of the total while the least common score was 6 constituting 10% of the total.

Ki-67: 40 cases were positive out of the fifty cases and 10 cases were negative for Ki-67 constituting 80% and 20% respectively. The percentage of positive cells varied between 3% to 84%. The staining intensity varied from moderate to strong with 26 cases showing strong staining intensity.

Correlation of Gleason's grade with Ki-67 expression: Of the 16 cases of most common pattern (5+4), 13 were positive for Ki-67 and 3 were negative for Ki-67.Thus showing an increase in positivity with increase in grade but it was not statistically significant (TABLE 1).

Correlation of Gleason's score with Ki-67 expression: Majority of patients of Gleason score 7-8 showed positivity for Ki-67(79.2%). It was observed that with increase in the score the number of cases showing positivity also increased but it was not statistically significant (p value=0.989) (TABLE 2).

p53: Out of the total 50 cases p53 was positive in 29 cases and negative in 21 constituting 58% and 42% respectively. The percentage of cells positive varied between 5% and 80%.The staining intensity varied from mild to strong with 18 cases showing moderate intensity.

Correlation of Gleason's grade with p53 expression: Of the 16 cases of most common pattern (5+4), 10 were positive for p53 and 6 were negative for p53. It was observed as the grade increased p53 positivity also increased but it was not statistically significant (TABLE 3).

Correlation of Gleason's score with p53 expression: Majority of patients of Gleason's score 7-8 showed positivity for p53 (62.5%). It was observed that with increase in the score the number of cases showing positivity also increased but it was not statistically significant (p value=0.647) (TABLE 4).

Combined Ki-67 and p53 expression: Out of the 50 cases, the majority of patients showed combined positivity for both Ki-67 and p53 constituting 48% of the total.

Correlation of Gleason's grade and score with combined Ki-67 and p53 expression: Out of the 16 cases of grade (5+4), combined Ki-67 and p53 positivity was seen in 12 cases (75%) (TABLE 5).Of the all cases of Gleason score 7-8, 11 cases showed both Ki-67 and p53 positivity but no statistically significant correlation was seen with increase in grade (p value = 0.648) (TABLE 6).

Thus, it was observed that Ki-67 and p53 have got a direct correlation with the Gleason grade and Gleason scoring but in the present study it was not statistically significant.

Table 1: Correlation of Ki-67 expression with Gleason grade.

Gleason Grade	Ki-67 –VE CASES(%)	Ki-67+VE CASES(%)	TOTAL (%)
4+4	1(9)	10(91)	11(100)
3+3	1(20)	4(80)	5(100)
3+4	3(33.3)	6(66.7)	9(100)
5+4	3(18)	13(82)	16(100)
5+5	1(20)	4(80)	5(100)
4+3	1(25)	3(75)	4(100)
TOTAL	**10(20)**	**40(80)**	**50(100)**

Table 2: Correlation of Gleason score with Ki-67 expression.

GRADING (According to score)	Ki- 67 –VE cases (%)	Ki-67 +VE cases (%)	TOTAL (%)
Gleason score 2-6 (Low Grade)	1 (20)	4 (80)	5(100)
Gleason score 7-8 (Intermediate Grade)	5(20.8)	19(79.2)	24(100)
Gleason score 9-10 (High Grade)	4(19.0)	17(81.0)	21(100)
TOTAL	**10(20)**	**40(80)**	**50(100)**

Table 3: Correlation of p53 expression with Gleason grade.

GLEASON GRADE	p53 –VE CASES(%)	p53+VE CASES(%)	TOTAL (%)
4+4	5(45.4)	6(54.6)	11(100)
3+3	3(60)	2(40)	5(100)
3+4	3(33.3)	6(66.7)	9(100)
5+4	6(37.5)	10(62.5)	16(100)
5+5	3(60)	2(40)	5(100)
4+3	1(25)	3(75)	4(100)
TOTAL	**21(42)**	**29(58)**	**50(100)**

Table 4: Correlation of Gleason score with p53 expression.

GRADING (According to score)	p53 –VE CASES (%)	p53+VE CASES (%)	TOTAL (%)
Gleason score 2-6 (Low Grade)	3(60)	2 (40)	5 (100)
Gleason score 7-8 (Intermediate Grade)	9 (37.5)	15 (62.5)	24 (100)
Gleason score 9-10 (High Grade)	9 (42.9)	12 (57.1)	21 (100)
TOTAL	**21 (42)**	**29 (58)**	**50 (100)**

Table 5: Gleason grade correlation with both p53 and Ki-67 expression.

GLEASON GRADE	BOTH p53 & Ki-67 -VE(%)	p53-VE AND Ki-67 +VE(%)	p53+VE AND Ki-67-VE(%)	BOTH p53 & Ki-67 +VE(%)	TOTAL(%)
4+4	0(0)	4(36.5)	2(18.1)	5(45.4)	11(100)
3+3	1(20)	2(40)	1(20)	1(20)	5(100)
3+4	2(22.2)	2(22.2)	2(22.2)	3(33.4)	9(100)
5+4	2(12.5)	2(12.5)	0(0)	12(75)	16(100)
5+5	0(0)	3(60)	0(0)	2(40)	5(100)
4+3	0(0)	3(75)	0(0)	1(25)	4(100)
TOTAL	**5(10)**	**16(32)**	**5(10)**	**24(48)**	**50(100)**

Table 6: Gleason score correlation with both p53 and Ki-67 expression.

GRADING (According to score)	p53-VE & Ki- 67 –VE cases (%)	p53 –VE & Ki-67 +VE cases (%)	p53 +VE & Ki-67 –VE cases (%)	p53 +VE AND Ki-67 +VE cases (%)	TOTAL
Gleason score 2-6 (Low Grade)	1 (20)	2 (40)	0(0)	2(40)	5(100)
Gleason score 7-8 (Intermediate Grade)	1(4.2)	8(33.3)	4(16.7)	11(45.8)	24(100)
Gleason score 9-10 (High Grade)	3(14.3)	6(28.6)	1(4.8)	11(52.4)	21(100)
TOTAL	**5(10)**	**16(32)**	**5(10)**	**24(48)**	**50(100)**

Discussion

Prostate carcinoma is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide. Now-a-days more patients are diagnosed at earlier stages. Increased early detection of the disease is due to increased availability of PSA measurement and also because of using other diagnostic methods such as cystoscopy, transurethral ultrasonography, biopsy and tumor markers. Various markers such as p53 and Ki-67 are expressed immunohistochemically in prostate cancer. Mutations of p53 tumor suppressor gene can result in uninhibited cellular growth while Ki-67 proliferation marker provides a reliable index of cellular expression and expression of both reflect poor prognosis in carcinoma patients.[13,10]

The present study showed that prostate carcinoma usually affects the elderly age group. Similar results have been seen in studies conducted by Cartar et al and Malti et al

who demonstrated that prostate cancer is more common in men > 65 years.[14, 15]

Gleason scoring was done and the most common score was 9, which was followed by 7 occurring in 32% and 26% of cases respectively. Similar results have been demonstrated by Petrescu et al and Madani et al who in their respective studies showed Gleason score of 8-10 in 43% and 51% of cases respectively.[16, 17]

p53 positivity was found in 29 cases (58%) while p53 negativity was found in 21 cases (42%). Percentage positive cells varied from 5-80% and exhibited mild, moderate and strong staining intensity. Moul et al and Sasor et al have found a higher p53 expression of 69.1% and 62.2 % respectively whereas Madani et al demonstrated a positive p53 expression in the range of 48%- 68% expression in high grade prostate cancer.[17, 18, 19]

Although expression and intensity of p53 increased with increase in grade; no statistically significant correlation was found between the two. This was also reflected by Sasor et al and Yaman et al who have statistically proven that there is no significant difference between the presence of p53 in low and high grade tumor.[19, 20] On the other hand, Kallakury BV et al and Petrescu et al have demonstrated a positive correlation between p53 immunopositivity and higher Gleason grades with expression of 21% and 39% respectively.[21, 16]

Ki-67 positive cells varied from 3-84% and moderate and strong staining intensity was found in 80% of cases in the present study. Similar higher positivity and strong staining intensity was also recorded by Madani et al, Thompson et al and Zhong et al in 71.4%,76.0% and 67.76% of cases respectively whereas a very high Ki-67 expression of 93% was observed by Makarewicz et al.[17, 22, 23, 24]

Out of the 32 cases of Gleason's score of 8 and above, Ki-67 expression was noted in 81% cases while 78% cases of Gleason's score 6 and 7 showed Ki-67 expression. Thus, showing increase in Ki-67 positivity with increase in grading but it was not statistically significant.

Madani et al also observed that Ki-67 expression increased from 62.2% to 88% as the grade increased.[17] Marian Sulik et al and Bettencourt et al have also stated significant correlation between immunopositivity of Ki-67 and higher Gleason grade.[25,18]Although Munroz et al have not found a statistically significant difference between Ki-67 expression and higher Gleason grade.[26]

The present study revealed 48% cases (24/50) exhibiting positivity for both of these markers. In a study done by Madani et al high Ki-67 expression was observed in 71 % cases of prostate cancer while p53 expression was noted in 42.9 % cases.[17] Moul et al showed Ki-67 expression in 38.3% of cases and p53 expression in 69% of cases.[18]

Out of the 32 cases of Gleason score 8 and above, combined Ki-67 and p53 expression was noted in 46% of cases while 50 % cases of Gleason score 6 and 7 showed combined p53 and Ki-67 expression. A study by Rashed et al demonstrated a positive relationship between the expressions of Ki-67 and p53 in patients with low grade prostate cancer.[29]

Borce et al have also showed that accumulation of p53 had a special correlation with patients survival. The presence and activity of p53 was greatly associated with the cell proliferation marker Ki-67 and the level of p53 activity was an important independent prognostic factor that was inversely associated with patient survival.[19]

Moul et al have also recommended the clinical use of Ki-67 and p53 immunohistochemical protein expression in the primary tumor as combined predictors of disease progression.[18]

In the present study antibodies were not employed to find an association between benign proliferative or in-situ lesions. Various studies Indian and abroad have documented that immunoexpression of p53 and ki-67 is up regulated in cancerous tissue as compared to benign prostatic ones such as nodular hyperplasia , adenosis and stromal hyperplasia and in situ lesions such as prostatic intraepithelial lesion (PIN).

While many researchers have documented the rate of immunoexpresssion of p53 in benign tissue to be low as 2 % to 10 %[27,28]; there are many (notably amongst them Petrescue A et al) who have reported a lack of immunoreactivity in all such cases. [16] The same work by Petrescue A also documented that the immunoexpression in PIN (in-situ lesion) especially the high grade (HPIN), as the insitu lesion moves from low to high; the expression increases. Similar findings have been recorded by researchers worldwide while studying Ki-67 expression, which is significantly low in benign prostatic conditions and PIN as compared with the cancerous tissue.[29]

This leads to the hypothesis whether the occurrence of p53 mutations and Ki-67 expression in prostatic carcinoma is an early event.[16, 27]

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Competing Interests
None Declared

Conclusion
Ki-67 and p53 were performed on a small cohort of Punjabi population (North India) which showed an increase in the expression of these markers with increasing grade and score. Hence, immunostaining with both these markers should be done in all cases of prostate carcinoma as these markers allow identification of tumors with a higher rate of cell growth, allowing the development of prognostic factors and new targeted therapeutic strategies for increased survival in these patients**.**

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