Original Article

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A Study of p53 immunostaining in prostate carcinomas: Correlation with Gleason's score

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ABSTRACT

Background: Prostate cancer is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide. In view of the above, early diagnosis and effective treatment of the disease are immensely important. The increasing number of options for the treatment of prostate cancer has made the prognostic evaluation of the disease even more important. P53 is a tumor suppressor gene characterised by a highly proliferative pattern and an aggressive behaviour. The Objectives was to study the immunostaining patterns of p53 in prostate cancers and to compare the results with Gleason's score.

Methods: Fifty cases of histopathologically proven prostate carcinomas diagnosed on needle biopsies and transurethral resection specimens was studied in JSS medical college and hospital, mysore for a period of 3 years and histopathological grade was assessed using Gleason grading system. Immunohistochemistry (IHC) for p53 was done on paraffin embedded wax sections.

Result: p53 staining was positive in 47(94%) cases out of 50 cases, three (6%) cases were negative. Although there was an increase in positive p53 staining with increased Gleason's score, it was not statistically significant ('p' value = 0.068).

Conclusion: p53 is a tumor suppressor gene, that express high proliferative pattern. It can be used as a prognostic factor. The immunoreactivity of p53 marker with increased tumor grade can benefit patients with appropriate targeted treatment and increase their survival period.

Keywords: *Prostate Cancer, Gleason's Score, p53.*

Introduction

Prostate cancer is the second most common cause of cancer and the sixth leading cause of cancer death among men worldwide. The mortality rates are highest in the Caribbean and lowest in South Central Asia, but this partly reflects varying data quality worldwide. Prostate cancer is strongly related to age with the highest mortality rates being in older males. Rise in the incidence of the prostate cancer has partly attributed with screening of prostate specific antigen (PSA) level. [1] Prostate cancer is not only significant for its lethality but also for the extremely high morbidity associated with it.[2]

Patients generally do not experience symptoms during early stage and are unlikely to seek medical help until the disease has progressed. Thus, prostate cancer is acknowledged as a major health problem and with the advent of screening tests like digital rectal examination(DRE) and prostate specific antigen test, more patients are being diagnosed in earlier stages. With delay in early diagnosis of the low-grade tumor, the quality or length of patient's life is not significantly changed, but a high-grade tumor in a young person might spread quickly and lead to the patient's death within two years. [3]

Now-a-days, prostatic needle biopsies and transurethral resected specimens are being increasingly used to diagnose prostatic carcinomas. Histopathologically, once the diagnosis of adenocarcinoma is made, it is graded using Gleason scoring system. Gleason system is one of the best prognostic predictor[4] recommended by World health organization and very acceptable by majority of urologists and radiotherapists. The Gleason grading system, named after Donald F. Gleason, is a unique histopathological method for grading prostate cancer based solely on the tumor architecture. [4,5] It is important preoperative predictor of the behavior of prostate cancer and is used to help in making decisions about treatment for localized prostate cancer.[6,7] It is also used to predict relapse in patients receiving hormone therapy for bone metastasis of prostate cancer.[8] But recent studies using various biomarkers have proved that many prostatic carcinomas have been over graded or under graded using Gleason scoring system.[5] This may be attributed to its subjective nature and has aroused the need to search for novel markers which are more objective and can predict the behavior of prostatic carcinoma[5]

P53 originally referred to a 53-Kilodalton Phosphoprotein, the product of a 20 – Kilobase gene on short arm of

chromosome 17. It plays critical role as a cancer suppressor**.** p53 is involved in the regulation of cell cycle, causing cell cycle arrest at G1 phase and in certain cell types precipitating apoptosis.[9] The importance of p53 in the pathogenesis of prostatic adenocarcinoma was first postulated by Rubin SJ et al[10] and Isaacs WB et al[11], who demonstrated mutations of p53 gene in prostate cell lines and in primary human prostatic adenocarcinoma. Many studies have suggested a significant association between p53 immunoreactivity and prostate carcinomas characterised by a highly proliferative pattern and an aggressive behaviour.[12,13] A good correlation has been found between the detection of mutations at the molecular level and the over expression of the protein as detected immunohistochemically.[14]

Despite improvements in early detection of prostate cancer as a result of DRE and PSA screening, we still lack molecular markers to effectively distinguish patients with high risk of disease progression from the indolent majority. Considering the proven correlation between Gleason's grading and prognosis of prostate cancer, p53 study is undertaken to investigate the frequency of expression of the marker in prostate cancer and its probable relation with Gleason's score.

Materials and Methods

In this study, fifty cases of Prostate carcinomas diagnosed on needle biopsies and transurethral resection specimens were studied in JSS medical college and hospital, Mysore for a period of 3years. Ethical committee clearance was obtained. The patient's medical records were reviewed to obtain patient's clinico-pathological parameters, including age at diagnosis, pretreatment sPSA values, digital rectal examination findings, type of procedure, ultrasonography and CT scan findings. All surgically removed prostate carcinoma tissues was fixed in formalin followed by paraffin embedding and stained with haematoxylin and eosin. Sections were studied and histological diagnosis was given according to WHO classification. All cases were assigned Gleason score by Gleason grading system.

IHC for p53 was done on 4 µm thick paraffin embedded wax sections on poly-l lysine coated slides. Antigen retrieval was done in tri sodium citrate buffer at pH 6. p53 antibody (Novocastra Code No RTU-p53-DO7) was used for p53 antigen by one step horseradish peroxidase (HRP) polymer method . A section from a poorly differentiated breast carcinoma was taken as positive control whereas sections treated with tris-buffer solution instead of primary antibody was used as negative control. Strong brown nuclear immunoreactivity was considered as positive staining.

The immunoquantification was performed using percentage of tumor cells that react with the antibody. Each slide was evaluated at x40 magnification in order to find areas with maximum positive cells. Then these areas were examined at x400 magnification and the percentage of positive cells to total cells was calculated. At least 500 cells were counted, and only the cells that were definitely positive was considered.

A semiquantitative scoring system was employed to assess the level of p53 reactivity. Grade 0 was assigned when no staining was observed, grade1 when <10% of tumor cell nuclei were reactive, grade 2 when >10% but <33% of the nuclei stained, grade 3 for >33% of nuclei were positive.

Data was analysed using Epi-info statistical software. Univariate analysis was done and expressed in mean and percentages. Bivariate analysis was done using Spearman's correlation coefficient, to find out the strength of association between histopathological report results and Gleason's score. The association of p53 as a categorical variable was determined by chi square test. Results were interpreted at alpha error level of 5%. p value of <0.05 was considered as statistically significant.

Result

In the present study, the age group of patients ranged from 44 to 86 years, with a mean age of 69.9 years. Patients predominantly presented with acute urinary retention (AUR) & lower urinary tract symptoms (LUTS). Digital rectal examination was abnormal in 45% of cases. 60% patients had grade II prostatomegaly on ultrasonography. All cases displayed features of Acinar Adenocarcinoma (Ordinary type) of which glandular pattern (84%), followed by cribriform (56%) were common patterns. Majority (70%) had poorly differentiated adenocarcinoma followed by moderately-poorly differentiated (24%) [Table-1] No cases of well differentiated adenocarcinoma was encountered during the study period. Perineural invasion (PNI) was seen in 48% of the cases. One case (2%) showed metastasis to bone which was picked up by Positron Emission Tomography (PET).

With p53 staining, fourth seven (94%) cases out of 50 cases showed positive staining for p53, three (6%) cases were negative. Based on tumor differentiation 15 (30%) were moderately differentiated and 35 (70%) were poorly differentiated. None of them were well differentiated. Seven (14%) cases were graded 1 (1-10%) of which four were moderately differentiated and three were poorly differentiated. 14 (28%) cases were graded 2 (10-33%) in which five were moderately differentiated and nine were poorly differentiated and 26 (52%) cases were graded 3 (>33%), of which four were moderately differentiated and 22 were poorly differentiated . [Table -1, Figure 1,2,3]

Table 1: Frequency of p53 in relation to differentiation and Gleason grade.

p53	Gleason Grade			
	Moderately differentiated	Poorly differentiated	Frequency	Percentage (%)
0	2	1	3	6.0
1-10%	4	3	7	14.0
10-33%	5	9	14	28.0
>33%	4	22	26	52.0
Total	**15**	**35**	**50**	**100.0**

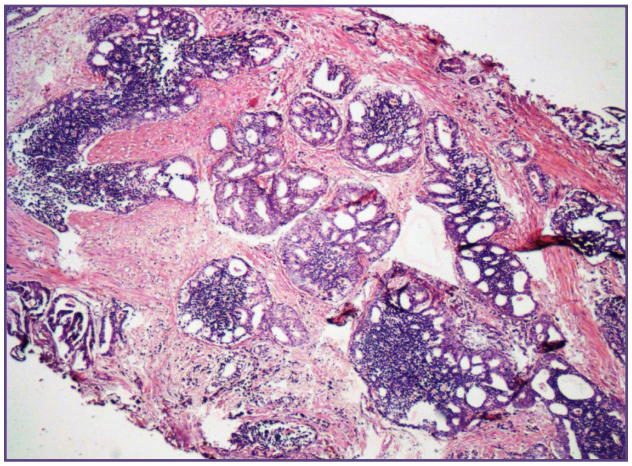

Fig. 1: Photomicrograph showing poorly differentiated adenocarcinoma with tumor cells arranged in cribriform pattern with lymphocytic infiltration. Gleason's score 4+4=8 (H&E, x100).

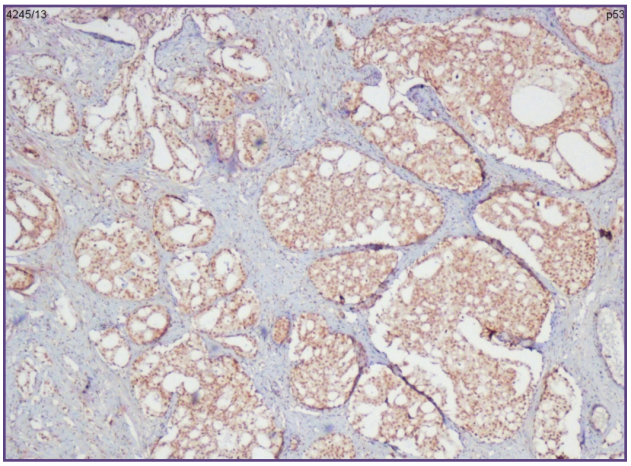

Fig. 2: Photomicrograph showing poorly differentiated adenocarcinoma with 3+ positivity for p53 immunostain. Gleason's score 4+4=8 (p53, x100).

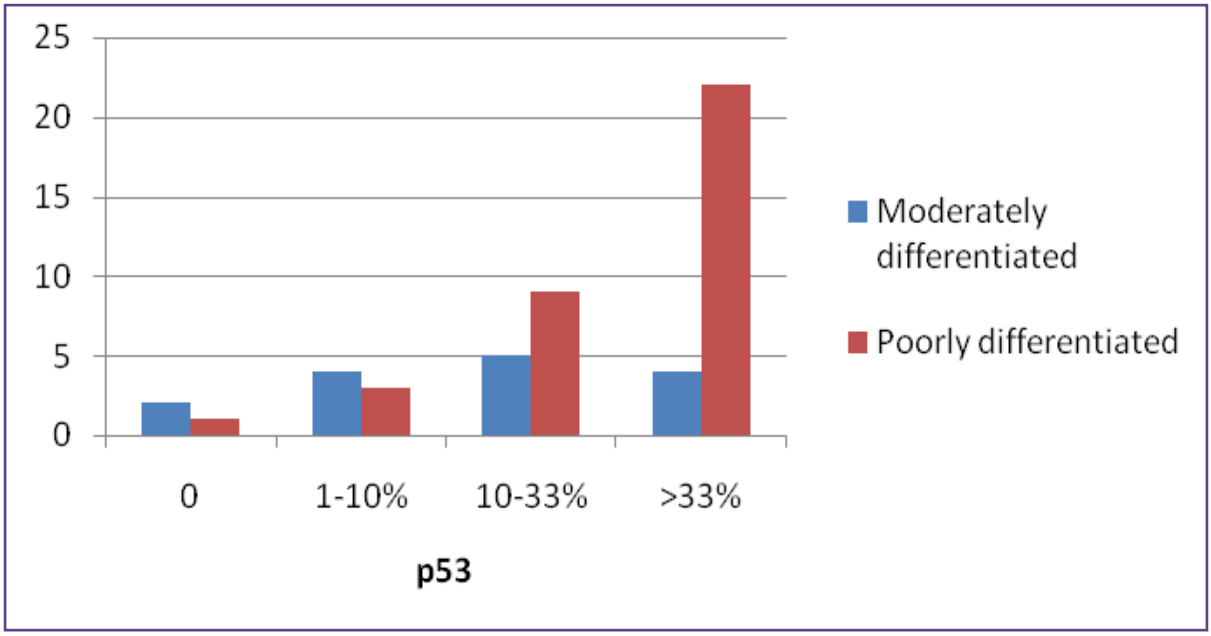

Fig. 3 : Comparison of results of p53 and Gleason's score among study samples.

Discussion

In our study, majority (70%) of tumors were poorly differentiated adenocarcinoma followed by moderately differentiated adenocarcinomas (30%). Whereas, similar studies done by Madani SH et al[3] (51%), Shirley SE et al[15] (60%), Catalona WJ et al[16] (60%) have also reported majority of prostatic adenocarcinomas are poorly differentiated with Gleason score between 8-10. On the contrary, Chiusa L et al[17], Petrescu A et al[9] (56.6%) have reported moderately differentiated adenocarcinoma (Gleason score 5-7) are the predominant type. The possible contributing factors for this differentiation difference might be genetic, environmental, racial, dietary factors or inter-observer variability in Gleason scoring or the authors who have still considered Gleason score 7 to be of moderately differentiated grade.

p53 over expression has been investigated independently in a large number of different malignancies for their potential value as a prognostic marker. Mutation of the p53 tumor suppressor gene is a common genetic alteration in malignant human tumors and can be immunohistochemically detected.[9] In our study, Out of 50 cases, 47(94%) cases showed strong nuclear positive staining for p53. Only three (6%) cases were negative. In the literature, the range of incidence of p53 positive immunoexpression in prostate cancers has been reported ranging from 4 to 61%.[19] Most of this variation are attributed to methodological differences in tissue sampling, antibody clone used and scoring.

Although there was an increase in positive p53 staining with increased Gleason's score, it was not statistically significant ('p' value = 0.068) in our study. The results were correlated with studies done by Visakorpi T et al[12], Bookstein R et al[20], Shurbaji MS et al[21], Grignon DJ[22] et al, Cappello F et al[23], Petrescu A et al.[9] According to them, there is significant association between p53 protein over expression and increased Gleason score. These results strongly imply that p53 mutations play a role in the pathogenesis of a subset of prostate cancers. But the precise molecular role played by the over expressed p53 protein in mediating oncogenesis in prostate epithelium remains to be determined. In contrary, study by Lin JT et al[13], Madani SH et al[3], had no statistically significant correlation between p53 positivity and increased Gleason's score and have said formalin fixation reduces expression of p53.

Whereas, Borre et al[24] have showed the accumulation of p53 have a special correlation with patients survival. The presence and activity of p53 was greatly associated with the cell proliferation marker (MIB-1) and the level of p53 activity was an important independent prognostic factor that was inversely associated with patient survival. But Sasor et al[25], showed that there is no significant difference between the presence of p53 in low and high grade tumors but there is only a positive relationship between the expressions of Ki-67 and p53 in patients with low-grade prostate cancer. Also, he has said that increased histologic grade and presence of metastases suggest that p53 expression may be linked to the tumor behavior.

Bookstein R et al[20], showed abnormal nuclear p53 expression may be an early event in prostatic carcinoma progression, Where as, Schlomm T et al[18] and Visakorpi T et al[12] have reported low frequencies of p53 positivity by immunohistochemistry and poor prognosis.

Conclusion

To conclude p53 is a tumor suppressor gene defines a small subgroup of highly malignant tumors. p53 immunoreactivity in prostate carcinomas are seen with high proliferative pattern and suggest the aggressive behavior of tumour. Also immunoreactivity of p53 marker with increased tumor grade can benefit patients with appropriate targeted treatment and increase their survival time. It can be used as prognostic indicator but further studies are required with more number of cases to determine their biologic role and progression of disease in prostate cancer.

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