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SIGNIFICANCE OF P53 AND KI67 IN BENIGN AND MALIGNANT LESIONS OF PROSTATE DIAGNOSED BY HISTOPATHOLOGY AND ITS CORRELATION WITH GLEASON'S GRADING SYSTEM: A TERTIARY CARE HOSPITAL BASED STUDY AT RIMS RANCHI.

Pathology

Dr. Santosh Kumar Post Graduate Student Department Of Pathology Rajendra Institute Of Medical Sciences Ranchi

Dr. Anshu Jamaiyar* Associate Professor Department of Pathology Rajendra Institute Of Medical sciences Ranchi *Corresponding Author

ABSTRACT

Background: Prostate cancer is a major health problem throughout the developed world. Tumor grade is one of the most important prognostic factors of prostate cancer. At present, adequate prognostic markers for prostate cancer progression are still lacking, in spite of intensive investigation. Accordingly, we will study the role of immunohistochemical (IHC) .expression of p53 and Ki67 as a prognostic factor in carcinoma prostate and correlate their expression with Gleason's grade.

Method : Prostate specimens collected at Deptt. of Pathology, RIMS, Ranchi from July,2017 to October,2018 will be studied prospectively.Prostate fragments will be fixed in 10% formalin, paraffin- embedded, sectioned and standard H and E stained sections will be studied under light microscope and classified into benign and malignant lesions. Carcinoma cases were histologically graded according to Gleason's grading system, and Gleason's score was noted (well differentiated 2–4, Moderately differentiated 5–7, poorly differentiated 8–10).

Result: A total 50 (100%) study participants were included in the study. The age of the study participants varied between 60 years to 83 years with mean (SD) of 70 (±5.08) years. The median age was 70.5 years. Histopathological diagnosis of the study participants were- Benign prostatic hypertrophy -4(8%), Malignant prostate lesion- 43(86%), Basal cell hyperplasia -1(2%), Adenomatous hyperplasia-1(2%) and atrophy of prostate – 1(2%). The tumors were divided into five groups regarding the percentage of Ki-67 positive cells. Cases in which the percentage of stained cells was ≤2% were considered negative. Cases with Ki-67 index of ≤25% were considered 1+, 26–50% as 2+, 51–75% as 3+ and 76–100% as 4+. Immunohistochemical staining of p53 was done and level of p53 reactivity was 0 – 7(14%), 1- 13(26%), 2-15(30%) and3- 15(30%) respectively.

Conclusion : From the present study, it will be concluded that frequency of expression of both p53, a tumor suppressor protein, and Ki-67, a cell proliferation marker is significantly up-regulated in malignant lesions as compared to benign lesions.

KEYWORDS

Immunohistochemistry , Benign Prostatic Hyperplasia , Carcinoma Prostate , Gleason grade

INTRODUCTION:

Prostate cancer is a major health problem throughout the developed world. Tumor grade is one of the most important prognostic factors of prostate cancer. At present, adequate prognostic markers for prostate cancer progression are still lacking, in spite of intensive investigation. Accordingly, we will study the role of immunohistochemical (IHC) expression of p53 and Ki67 as a prognostic factor in carcinoma prostate and correlate their expression with Gleason's grade. Prostate cancer is the second most frequently diagnosed cancer and the fifth leading cause of cancer death in males.[1]Incidence increasesfrom 20% in men in their fifties to approximately 70% in men between the age of 70 and 80 years.[2] Prostate cancer is not only significant forits lethality but also for the extremely high morbidityassociated with it. Nowadays, more patients are diagnosed at earlier stages, due to increased availability of prostatic-specific antigen (PSA) measurement and other diagnostic methods. With delay in 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 2 years. The absence of prognostic information has also led to significant "overtreatment" of patients whowould otherwise require only conservative management. Prognostic factors are divided into clinical and biological groups. Clinical factors are obtained using blood tests, radiological and microscopic evaluation of biopsies. Biological factors are other categories of prognostic factors.[4] Grade and stage, the traditional prognostic markers, are useful, but for individual patients, it is difficult to predict the outcome.With recent advances in molecular biology, the concept of oncogenes, tumor suppressor genes has dominated basic science research of tumorigenesis. Evaluation of these genes and their protein products may provide new prognostic markers, with p53 and Ki-67 gaining special attention.[5] In some studies, the incidence of p53 has been associated with higher grades of prostatic tumors and worse prognosis of the disease.[6] Although another studies revealed different results, nuclear staining for p53 was positive in at least a subset of prostatic cancers and there is still a discrepancy in the frequency of p53 mutations in carcinoma prostate and on its prognostic role.[4,7] Ki-67 index is higher in carcinoma than hyperplasia and stillhigher in metastatic than non-metastatic cases, thus an increased Ki-67 index may indicate a poor prognosis of disease.[4] However, its role as an independent prognostic marker among patients with prostate carcinoma is still controversial Considering the proven correlation between Gleason's grading and prognosis of prostate cancer, along with proposing of p53 (tumor suppressor protein) and Ki-67 (cell proliferation marker) as prognostic factors; this study will be performed to study the frequency of these markers expression in prostatic cancer and their probable relation with Gleason's grading The **Gleason grading system** is used to help evaluate the prognosis of men with prostate cancer using samples from a prostate biopsy. Together with other parameters, it is incorporated into a strategy of prostate cancer staging which predicts prognosis and helps guide therapy. A Gleason score is given to prostate cancer based upon its microscopic appearance.[1] Cancers with a higher Gleason score are more aggressive and have a worse prognosis. Pathological scores range from 2 through 10, with higher number indicating greaterrisks and higher mortality. A total score is calculated based on how cells look under a microscope, with half the score based on the appearance of the most common cell morphology (scored 1—5), and the other half based off the appearance of the second most common cell morphology (scored 1—5). These two numbers are then combined to produce a total score for the cancer.

Methodology : Prostate specimens collected at Deptt. of Pathology, RIMS, Ranchi from July,2017 to October,2018 will be studied prospectively.Prostate fragments will be fixed in 10% formalin, paraffin- embedded, sectioned and standard H and E stained sections will be studied under light microscope and classified into benign and malignant lesions. Carcinoma cases were histologically graded according to Gleason's grading system, and Gleason's score was noted (well differentiated 2–4, Moderately differentiated 5–7, poorly differentiated 8–10). Associated prostatic tissue changes like tumor invasion, prostatic intraepithelial neoplasia (PIN), prostatitis and others if any, were also analyzed. Special stains like van Gieson, Periodic acid-Schiff, Masson's trichrome and reticulin were employed whenever required for histopathological diagnosis.

Inclusion criteria. 1. All Benign prostatic hyperplasia cases. 2. All Prostatic Neoplasms.

Exclusion Criteria

1. Degraded /Autolysed samples.
2. Less than 3 high power field area for analysis in any of the regions.

Data Analysis Data will be analyzed using frequency, percentage, mean and standard deviation. The correlation will be done using Carl Pearson correlation coefficient, T test and Chi square test as applicable. Values will be presented as charts,bar etc.as required. Results will be

discussed and correlated with the analysed literatures and conclusion will be drawn keeping in mind about the limitations of the study.

RESULTS : A total 50 (100%) study participants were included in the study.The age of the study participants varied between 60 years to 83 years with mean (SD) of 70 (±5.08) years. The median age was 70.5 years. (Table 1)

Table 1 Baseline parameters of study participants. (n=50)

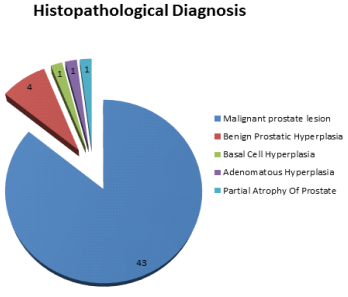
S.No.	Profile of study participants	N=50 N(%)
1.	Age group (in years) 60 -70 71-80 >80	 25(50) 24(48) 1(2)
2.	BMI <18.5 18.5-22.9 23-24.9 >25	 3(6) 16(32) 9(18) 22(44)
3.	Residence Rural Urban	 36(72) 14(28)

DIAGNOSIS Table 2 Histopathological diagnosis of the study participants. (n=50)

S.No.	Diagnosis	N=50 N(%)
1.	Histopathological diagnosis Benign prostatic hypertrophy Malignant prostate lesion Basal cell hyperplasia Adenomatous hyperplasia Atrophy of prostate	 04(08) 43(86) 01(02) 01(02) 01(02)

Histopathological diagnosis of the study participants were- Benign prostatic hypertrophy -4(8%), Malignant prostate lesion- 43(86%), Basal cell hyperplasia -1(2%), Adenomatous hyperplasia-1(2%) and atrophy of prostate – 1(2%).

Figure 1 : Pie chart showing distribution of study participants according to histopathological diagnosis. (N=50)



IMMUNOHISTOCHEMICALANALYSIS

A semiquantitative scoring system was employed to assess the level of p53 reactivity: 0 - was assigned when no staining was observed, 1 - when < 10% of tumor cell nuclei were reactive, 2 - when more than 10%, but < 33% of the nuclei stained, and 3 - if more than 33% of nuclei were positive. The tumors were divided into five groups regarding the percentage of Ki-67 positive cells. Cases in which the percentage of stained cells was ≤2% were considered negative. Cases with Ki-67 index of ≤25% were considered 1+, 26–50% as 2+, 51–75% as 3+ and 76–100% as 4+. Immunohistochemical staining of p53 was done and level of p53 reactivity was 0 – 7(14%), 1- 13(26%), 2-15(30%) and3-15(30%) respectively.

Level of p53 reactivity was 0 – 7(14%), 1- 13(26%), 2-15(30%) and3-15(30%).

Table 3: Immunohistochemical profile with p53. (n=50)

S.No.	**Immunohistochemistry**	**N=50 N(%)**
1.	**Immunohistochemical analysis with p53** 0 1 2 3	 7(14) 13(20) 15(28) 15(38)

Immunohistochemical staining of Ki67 was 1+ - 7(14%), 2+ - 13(26%), 3+ -15(30%) and 4+ -15(30%). (Table 4)

Table 4 Immunohistochemical profile with Ki67. (n=50)

S.No.	Immunohistochemistry	N=50 N(%)
1.	Immunohistochemical analysis with Ki 67 1+ 2+ 3+ 4+	 7(14) 13(26) 15(30) 15(30)

PSA levels (ng/ml) was <10 in 7(14%), 10-50 in 36(72%), >50 in 7(14%) study participants.(Table 5)

Table 5 PSA levels of study participants. (n=50)

S. No.	PSA value	N(%)
1.	PSA (ng/ml) <10 10-50 >50	 7(14) 36(72) 7(14)

Table 6 Gleason grading system of the study participants. [Malignant cases (n=43)]

S. No.	Gleason grading system	N(%)
1.	Gleason grading system Low grade prostate cancer (2-5) Intermediate grade prostate cancer (6-7) High grade prostate cancer (8-10)	 6(14) 34(79.1) 3(6.9)

Among 43 prostate cancer cases 6 were low grade 34 cases were intermediate grade and 3 cases were high grade.

Association of gleason grading with ki67

Table 7 Association between growth retardation and baseline characteristics. (N=43)

S. No.	Immunohisto chemical analysis	Gleason score grading			*p* value
		Low grade N(%)	Intermediate grade N(%)	High grade N(%)	
1.	Ki 67 2+ 3+ 4+	3 1 2(13.33)	10 14 10(66.66)	0 0 3(20)	0.006

Chi square test applied, p value <0.05 is significant

Association between immunohistochemical analysis with Ki67 and Gealson score grading was made with chi square test. It was observed from the analysis that intermediate grade and high grade of gleason scores was associated with higher (3+ or 4+ staining). This was found to be statistically significant. (p value =0.006) (Table 7)

Association of gleason grading with p53

Table 8 Association between growth retardation and baseline characteristics. (N=43)

S. No.	Immunohisto chemical analysis	Gleason score grading			p value
		Low grade N(%)	Intermediate grade N(%)	High grade N(%)	
1.	P53 1 2 3	 3 1 2(13.33)	 10 14 10(66.66)	 0 0 3(20)	0.006

Chi square test applied, p value <0.05 is significant

Association between immunohistochemical analysis with p53 and Gealson score grading was made with chi square test. It was observed from the analysis that intermediate grade and high grade of gleason scores was associated with higher (2 0r 3 level staining). This was found to be statistically significant. (p value =0.006) (Table 8)

Table 9 Correlation of gleason score between p53 and Ki67. (N=50)

S.No.	Immunohistochemical analysis	Correation coefficient Gleason score	P value
1.	Ki 67	0.839	0.00
2.	P53	0.839	0.00

Pearson correlation coefficient

Correlation was made between gleason scores and p53 , Ki 67 staining and it was observed that higher scores of gleason score were associated with higher levels of Ki67 and p53 staining.(Table 9)

DISCUSSION :

The present study was conducted with the objective to evaluate the immunohistochemical expression of ki 67 antigen and p53 protein in prostatic lesion and to correlate gleason's grading with p53 and ki67. A total 50 (100%) study participants were included in the study.The age of the study participants varied between 60 years to 83 years with mean (SD) of 70 (±5.08) years. The median age was 70.5 years. PSA levels (ng/ml) was <100 in 7(14%), 101-200 in 36(72%), >200 in 7(14%) study participants.

Histopathological diagnosis

Histopathological diagnosis of the study participants were- Benign prostatic hypertrophy -4(8%), Malignant prostate lesion- 43(86%), Basal cell hyperplasia -1(2%), Adenomatous hyperplasia-1(2%) and atrophy of prostate – 1(2%). Another study by mahadev KS et al[7] reported that Out of the 100 cases studied, all case were of acinar/usual adenocarcinoma-WHO type. **Immunohistochemical expression of ki67 antigen and p53** The tumors were divided into five groups regarding the percentage of Ki-67 positive cells. Cases in which the percentage of stained cells was ≤2% were considered negative. Cases with Ki-67 index of ≤25% were considered 1+, 26–50% as 2+, 51–75% as 3+ and 76–100% as 4+. Immunohistochemical staining of Ki67 was 1+ - 7(14%), 2+ -13(26%), 3+ -15(30%) and 4+ -15(30%). A semiquantitative scoring system was employed to assess the level of p53 reactivity: 0 - was assigned when no staining was observed, 1 - when < 10% of tumor cell nuclei were reactive, 2 - when more than 10%, but < 33% of the nuclei stained, and 3 - if more than 33% of nuclei were positive. Immunohistochemical staining of p53 was done and level of p53 reactivity was 0 – 7(14%), 1- 13(26%), 2-15(30%) and 3-15(30%) respectively. In a study done by Kaur H et al [8] reported that 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. Another study by Monroe E et al[9] reported similar findings- A total of 162 prostate biopsies taken from patients diagnosed for benign prostatic hyperplasia (BPH, n=49), low grade prostatic intraepithelial neoplasia (LGPIN,n=53),high grade prostatic intraepithelial neoplasia (HGPIN, n=25) and carcinoma (CAR, n=35), were studied.

Gleason score grading with p53 and ki67gleason grading score of study participants with minimum score of 4 to maximum score of 9. Gleason grading was low grade prostate cancer (14%), intermediate grade prostate cancer (79%) and high grade prostate cancer (7%). Association between immunohistochemical analysis with Ki67 and Gealson score grading was made with chi square test. It was observed from the analysis that intermediate grade and high grade of gleason scores was associated with higher (3+ or 4+ staining). This was found to be statistically significant. (p value =0.006). Similar results were found in a study by Kaur H et al [8] which reported that 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. Another study by mahadev KS et al[7] reported that 40 cases were moderately differentiated, 39 cases were high grade tumors and 21 cases were intermediate grade tumors Ki67 labelling index showed a proportionate increase with increase in GS and serum PSA which was statistically significant.[7] A study done by Monroe E et al[9] reported that the correlation between the immunolabeling for Ki-67 and the histological diagnosis showed highly significant differences between BPH and CAR,LGPIN and CAR and HGPIN and CAR,with no significant differences being found among the other groups. Analysis of the immunolabeling in luminal cells of non-invasive lesions showed an increase in accordance with the increase in the degree of histological lesion,the greatest percentage being obtained in the HGPIN lesions (88.0%), with significant differences among all the groups. However in a study done by Grover S K et al[10] contradicting results were found and the study reported that Expression of Ki 67 was higher in carcinoma than benign hyperplasia. There was no correlation between the ERβ status, Ki 67 expression & grade of tumor.

CONCLUSION

- Ki-67 and p53 were performed on a small cohort of North India which showed an increase in the expression of these markers with increasing grade and score.
- Statistically significant association was found between intermediate grade and high grade of gleason scores with higher (3+ or 4+) staining of Ki-67 and p53.
- Hence, immunostaining with both these markers in addition to gleason score grading 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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