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# Expression of p53 in Renal Cell Carcinoma and its Association with Histological Types, Grades and Pathological Staging

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## Abstract

**Background:** The pattern of overexpression of p53 in RCC and their correlation with the histological types, Grading and pathological staging can guide the oncologist for selection of the patients. **Objective:** The aim of the study was to observe the pattern of overexpression of p53 in RCC and their correlation with the histological types, Grading and pathological staging. **Methodology:** This cross-sectional study was carried out in the Department of Pathology at Dhaka Medical College, Dhaka, Bangladesh from January 2018 to December 2019. Here 50 cases were selected as per inclusion and exclusion criteria. Histopathological investigations were done. Information were recorded in a prepared proforma. **Results:** Increased p53 expression was seen in 40.0% of the histologic sections. There is a significant association of p53 expression with some demographic variables (age and betel quid chewing), the tumor grading, pathological staging and tumor subtype (Papillary and clear cell type). It was also found that p53 expression was more prevalent in nonconventional (Papillary type). The p53 expression was observed in association with the presence of bad prognostic markers, like high tumor grading and staging. **Conclusions:** The expression of p53 is significantly associated with a number of bad prognostic factors in renal cell carcinoma. *[Journal of National Institute of Neurosciences Bangladesh, January 2022;8(1): 57-61]*

**Keywords:** p53; renal cell carcinoma; histological types

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## Introduction

Renal cell carcinoma is the most common type of kidney cancer and the 6th most frequently diagnosed cancer in men and the 10th in women is represented by renal cell carcinoma[1]. In higher-income countries there is increasing incidence rates of RCC as because renal masses are more incidentally detected. Around 50.0% of patients with renal cell carcinoma (RCC) will succumb of the disease within 5 years of diagnosis[2]. Survival is most significantly correlated with clinical stage. Other variables such as histopathological grade, DNA ploidy and proliferation rate have also been associated with prognosis[3]. Tumor growth is determined by the balance between proliferation and apoptosis for tumorigenesis in RCC. Mutations or alterations in oncogenes or suppressor genes, also affect cell growth regulatory

systems. Wild-type p53 protein is involved in both cell-cycle arrests after DNA damage and apoptosis, but is also believed to be involved in mitotic checkpoint regulation[4]. Mutation of the p53 gene is the most common single mutation found in human cancer[5]. The presence of mutated p53 protein in tumors has been related to poor prognosis in several cancers such as lung, breast and prostate cancer[5].

Prognosis of RCC depends on tumor grade, stage, distant metastasis, renal vein invasion, p53 overexpression etc. Generally, the higher grade and advanced stage has poor prognosis. Prognostic factors are insufficient in determination of the outcome of disease. Although tumor grades and stages are conventional clinico-pathologic parameters and are known to be prognostic factors for RCC, there are also many controversial cases. Several biological and molecular parameters have been suggested as potential prognostic markers for RCC. It is difficult to predict any single factor for accurate prognosis. Other than this, the therapeutic weapons are limited in RCC and they permit only a limited improvement[6].

The tumor suppressor gene p53 has been implicated in the initiation and progression of a number of malignancies[7]. In patients with Li-Fraumeni syndrome, for example, germline mutations of p53 are associated with the development of multiple different malignancies[8]. In patients with colorectal carcinoma and glioblastoma, p53 abnormalities are associated with the progression from benign or low grade tumors to malignant or high grade neoplasms[9]. p53 abnormalities are so frequent and because loss of p53 function may be associated with the progression of malignancy, the present study was performed in order to characterize more precisely abnormalities on overexpression of the p53 gene which was identified as in immunohistochemistry in renal cell carcinoma[3]. p53 overexpression, have been proposed as a prognostic factor. Loss of p53 function leads to more aggressive and invasive phenotype in many human cancers[7].

Though the histological grade and tumor stage determines the most important prognostic variables for tumor progression, but they cannot predict accurately the behavior of most RCC9. In Bangladesh, study of p53 expression in RCC has not been evaluated properly. Overexpression of p53 can there by guide the oncologist for selection of those patients who are at high risk for progression of RCC and cancer recurrence. Therefore, they may be benefited from adjuvant treatment modality or targeted therapeutic strategy and the eventual use of neo-adjuvant therapy in developing countries like ours. The aim of the study was to observe the pattern of overexpression of p53 in RCC and their correlation with the histological types, Grading and pathological staging.

**Methodology**

This cross-sectional study was carried out at the Department of Pathology at Dhaka Medical College, Dhaka, Bangladesh over a period of two years from January 2018 to December 2019. Nonrandom purposive sampling method was followed. Patients of any age group of either sex (male and female) with histologically diagnosed renal cell carcinoma (RCC) of the kidney were selected as study population. Patient who received neo-adjuvant or adjuvant chemotherapy due to renal cell carcinoma or kidney tumors other than renal cell carcinoma were excluded from this study. During the collection of specimens, all relevant information were recorded systematically in a prepared proforma. All the cases were numbered chronologically and the same number was given to H and E as well as immunohistochemically stained slides. Paraffin embedded tissue block selection along with patient clinical information were collected from department of Pathology, Dhaka Medical College, BSMMU and a private laboratory of the Dhaka city. Routine tissue processing and routine H &E staining were done on all cases at the Department of Pathology at DMC. Immunostaining for p53 was done at Square Hospital, Dhaka. For immunohistochemistry staining 4-micrometer thick tissue sections were taken on Poly-L lysine coated slide from the paraffin blocks of tumor. Primary Antibody was FLEX Monoclonal Mouse Anti-Human p53 Clone NCH-38 Ready to use (LINK). Secondary Antibody was DAKO REALTM EnVision TM (HRP RABBIT/MOUSE) (ENV). The presence of p53 staining in colonic adenocarcinoma cells served as an internal positive control. Immunohistochemical Evaluation of P53 was done by Nuclear staining of p53 and was classified according to its immunoreactivity by two independent investigators on two separate occasions and scored as positive when more than 5% immunoreactivity of cancer cell and negative when less than 5% Immunoreactivity of cancer cell. p53 reported as positive and negative with no grading (Kabiri et al. 2006). After meticulous checking and rechecking all data were recorded in a predesigned data collection sheet. Continuous variables were expressed as mean ± SD and were compared between groups of patients by student's 't' test. Categorical variables were compared using a chi-square test or Fischer's exact test as appropriate, and were presented as absolute frequencies with percentages. All P values were two-tailed with

significance defined as p <0.05 at the level of 95% confidence interval (CI). All analysis was done using the SPSS 22 (Statistical Package for Social Sciences).

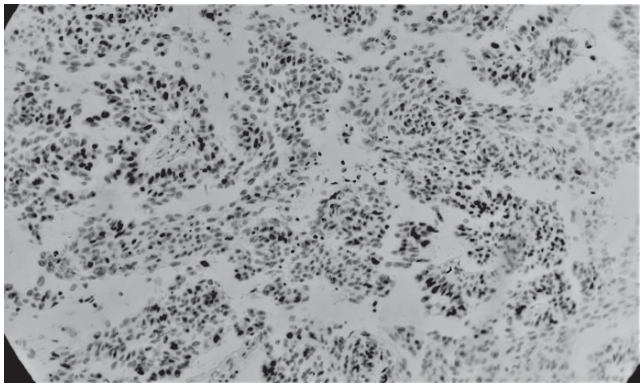

Figure 3.1: Example of p53 immunostaining in RCC (p53 expression, >5% )

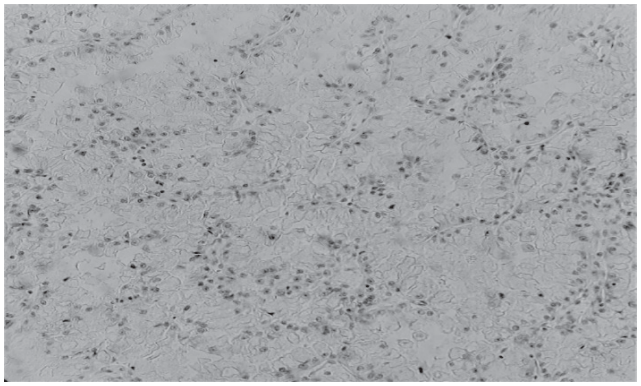

Figure 3.2: Example of p53 immunostaining in RCC (p53 expression, < 5%)

## Results

This cross-sectional study included 50 cases of renal cell carcinoma at the Department of Pathology in Dhaka Medical College. In all of Histopathologically diagnosed renal cell carcinoma cases, immunohistochemical staining was done. The association of age of the patients with p53 expression had shown that 39.5% patients with positive p53 expression were in ≤60 years age group and on the other hand, 42.9% patients who had positive p53 expression were in more than 60 years age group. The mean (±SD) age of the patients with positive p53 expression and negative p53 expression were 56.95 (±5.94) years and 50.57 (±12.26) years respectively. Statistically significant (p<0.05) difference was observed between age and p53 expression (Table 1).

The association of histologic type of the patients with p53 expressionhad shown that 27.0% clear cell, 75.0% papillary and 100.0% chromophobe type had positive p53 expression. Statistically significant (p<0.05) difference was observed between clear cell and papillary types with that of p53 expression (Table 2).

Table 1: Association of age and p53 expression (n=50)

| Age Group | p53 Expression | | P value |
|---|---|---|---|
| | Positive | Negative | |
| o ≤60 Years | 17(39.5%) | 26 (60.5%) | |
| o >60 Years | 3 (42.9%) | 4 (57.1%) | |
| Mean ± SD | 56.9±5.94 | 50.6±12.26 | 0.018[c] |

Unpaired t test was done to measure the level of significance.

Table 2: Association of histologic type and p53 expression (n=50)

| Histologic type | p53 Expression | | P value |
|---|---|---|---|
| | Positive | Negative | |
| Clear cell | 10(27.0%) | 27(73.0%) | 0.002 |
| Papillary | 9(75.0%) | 3(25.0%) | 0.007 |
| Chromophobe type | 1(100.0%) | 0(0.0%) | 0.400 |

Chi-square test was done to measure the level of significance.

The association of histopathological grade of the patients with p53 expression was measured and had found that 18.8% grade-1, 40.0% grade-2 and 77.8% grade-3 patients had positive p53 expression. Statistically significant (p<0.05) difference was observed between histopathological grade and p53 expression (Table 3).

Table 3: Association of histopathological grade and p53 expression (n=50)

| Histopathological grade | p53 Expression | | P value |
|---|---|---|---|
| | Positive | Negative | |
| Grade 1 | 3(18.8%) | 13(81.3%) | |
| Grade 2 | 10(40.0%) | 15(60.0%) | |
| Grade 3 | 7(77.8%) | 2(22.2%) | 0.015 |
| **Total** | **20(40.0%)** | **30(60.0%)** | |

Chi-square test was done to measure the level of significance.

The association of pathological staging of the patients with p53 expression were measured and had found that 17.4% T1, 52.4% T2 and 83.3% T3 patients had positive p53 expression. Statistically significant (p<0.05) difference was observed between pathological staging and p53 expression (Table 4).

Table 4: Association between pathological staging and p53 expression (n=50)

| Stage | p53 Expression | | P value |
|---|---|---|---|
| | Positive | Negative | |
| pT1 | 4(17.4%) | 19(82.6%) | |
| pT2 | 11(52.4%) | 10(47.6%) | |
| pT3 | 5(83.3%) | 1(16.7%) | 0.004 |
| **Total** | **20(40.0%)** | **30(60.0%)** | |

[a]Chi-square test was done to measure the level of significance.

**Discussion**

The relationship between renal cell carcinoma and p53 expression has not been well demonstrated. Till now the prognosis in renal cell carcinoma appears to be largely related to histopathologic grade and stage and the role of non-surgical options had limited value. In this study, expression of p53 was detected in 40% tumors. Other studies have reported this rate to be 20 to 30% cases[11]. The expression was relatively high in our cases. The p53 expression was evaluated by immunohistochemistry defined as more than 5% positively stained cell nucleus in this study. One explanation for the difference in expression of p53 might be because of the difference in the definition of positive staining and the antibody used. There were 27.0% clear cell, 75.0% papillary and 100.0% chromophobe type of RCC, which showed positive p53 expression.

It had been found that p53 expression was more frequent in nonconventional tumor subtypes that is papillary cell carcinoma. Thus, the higher rate of p53 expression in our case, can probably be explained by higher expression of p53 in papillary RCC among all non-conventional subtypes. Zigeuner et al[12] reported p53 overexpression in 11.9%, 27.3% and 70% of conventional (clear cell), chromophobe, and nonconventional (papillary) subtypes of RCC. Ferlay et al[2] also found 30.0% clear cell and 43% papillary renal cell carcinoma were positive for p53 expression. This finding is also supported by the study done by Noroozinia et al[5] and Ljungberg et al[6] who stated that overexpression of p53 was significantly more frequent in papillary subtype, when compared with conventional types like clear cell carcinoma. During analysis of p53 expression and type we found that, both clear and papillary subtypes had significant association. However, this finding is contrary to study done by others, where no relation was found[10,12]. Kabiri et al[13] didn't find any statistically significant association between conventional subtypes with that of p53 expression.

In this study, mean age of the patients was 53.12 (±10.61) with (male was 76% and female was 24%) male to female ratio of 3:1.7 that is similar to the study of Kabiri et al[13]. They found mean age of the patients was 52.64 years (SD: 13.49) with male to female ratio of 1.48 (59.7% was male and female was 40.3%). This is further supported by a similar study done by Lee et al[14]. There may be other confounding factors responsible for higher M: F ratio. The higher incidence of RCC in male may be due to the personal habit such as smoking.

The tumors in this study were grouped according to The Heidelberg classification of renal cell tumors[15]. It was observed that maximum (74.0%) patients had clear cell, followed by 24.0% patients who had papillary type and only 2.0% patients had chromophobe type of carcinoma. Ljungberg et al[6] in their study found 81.11% patients had clear cell, followed by 13.33% patients who had papillary and only 5.56 % patients had chromophobe type of carcinoma. In relation to the incidence of renal cell carcinoma (RCC), clear cell type patients were more in our study.

In this study, p53 immunoreactivity was more frequently observed in advanced tumors as reflected by tumor grading and pathological staging. Among the 50 patients, 32.0% were reported as grade-1, 50.0% as grade-2 and remaining 18.0% were reported as grade-3 renal cell carcinoma according to Fuhrman nuclear grading. It had found 18.8% grade-1, 40.0% grade-2 and 77.8% grade-3 patients were positive for p53 expression. Statistically significant ($p<0.05$) difference was observed between histopathological grade and p53 expression. Cho et al[9] stated that immunostaining for p53 was associated with tumor stage and grade and was an independent prognostic indicator for survival among patients with early stage renal tumors. In analysis of p53 expression and grade, Mombini et al[11] reported same. The observation of positive association suggests that p53 mutation might be a late event in tumorigenesis. This is opposed by observation of Noroozinia et al[5] and Zigeuner et al[12] who found no statistically significant difference in p53 expression in relation to tumor grade. So this is contrary to our finding.

In this study, 46.0% patients were in tumor stage pT1, 42.0% in pT2 and 12.0% were in pT3. 17.4% pT1, 52.4% pT2 and 83.3% pT3 patients were positive for p53 expression. Statistically significant ($p<0.05$) difference was observed between tumor stage and p53 expression. Zigeuner et al[12] found 40% T3 and 30.8% T2 patients were positive for p53 expression. Mutation of the p53 gene is the most common single mutation found in human cancer[16]. The strong correlation is seen in higher grade and advanced stage of RCC to indicate that p53 expression or mutation play an important role in the progression of RCC.

P53 positive expression was most frequently detected in high grade RCC patients (77.8%) than in low grade (18.8%+ 40.0%), ($p<0.05$). Negative expression was also found in 22.2% patients of higher grade of RCC. Higher grade with negative p53 expressions is

associated with decreased aggressiveness and requires lower aggressive therapy. In this present study, we observed 7(77.8%) p53 positive high grade RCC cases. They will be considered as high-risk patients with RCC and will need more aggressive therapy.

In this current study, we observed that, in pT3, 5(83.3%) cases were p53 positive and in pT2, 11 (52.4%) cases were positive, whereas in pT1, 4(17.4%) cases were p53 positive. Positive cases will be considered as high risk patients with RCC and will need more aggressive therapy. In pT2, there are 10 (47.6%) p53 negative cases and in pT1, 19 (82.6%) p53 negative RCC cases. They fall into lower stages and also p53 negative cases. Therefore, they are not the high risk cases. Therefore, they do not need higher aggressive therapy.

There was only 1(16.7%) higher stage RCC patient with negative expression of p53 found in this study. This is the controversial case. Other prognostic factors should be taken into consideration such as lymph node status, gene amplification etc. for their treatment. This present study found a significant association between p53 expression and bad prognostic factors in RCC. So these results validate and support previous studies demonstrating a correlation between p53 immunoreactivity and prognostic markers. This study could have been more effective if a greater number of RCC cases were included and follow up was done to see the progression of the disease and recurrence.

## Conclusion

The expression of p53 is significantly associated with a number of bad prognostic factors in renal cell carcinoma. Work is in progress to establish whether mutation of p53 can be blocked by gene therapy or biological therapy to induce a lesser aggressive RCC. Use of p53 expression is needed in Renal cell carcinoma of the kidney for the assessment of aggressiveness of the tumor as well as to see the prognosis of the cancer patients. By avoiding the more invasive procedure, like- open biopsy or partial nephrectomy, core biopsy may be done followed by p53 immunostaining to asses tumor biology and prognosis.

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