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Determining The Immunohistochemical Expression of P53 and its Role in Grading Urothelial Carcinoma

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Abstract:-

➢ ***Objective***

To establish the importance of immunohistochemical staining of P53 in grading of Urothelial Carcinoma.

➢ ***Methodology***

A retrospective cross-sectional study was carried out at the department of Histopathology, Lady Reading Hospital (Medical Teaching Institution), Peshawar, from August 2021 till February 2022. 97 Paraffin embedded blocks of Urothelial carcinoma along with clinical record of these patients, from January 2018 till December 2020, were retrieved from data bank of Histopathology department and Health management information system. Cutting of blocks and staining with Hematoxylin and Eosin (H&E) stain and P53 antibody was done. Expression of p53 was noted by two consultant pathologists. Nuclear immunoreactivity of strong intensity was considered positive, if present in more than 10% of tumor cells, and negative if either no staining or staining in less than 10% of tumor cells was noted. Statistical analysis (Pearsmann correlation) was used to determine the correlation among various variables.

➢ ***Results***

Out of 97 patients, the minimum age was 20 years while maximum age was found to be 90 years with mean ± standard deviation of 64 ±11 years. There were 86 (88.6%) male patients and 11 (11.3) female patients. 49(51%) cases were low grade Urothelial carcinoma whereas 48(49%) cases displayed high grade morphology. p53 was found positive in 63(64.95%) patients. Among positive cases, 45 cases were high grade and 18 were low grade Urothelial carcinoma.

➢ ***Conclusion***

P53 Positivity was seen in 64.95% patients with Urothelial carcinoma. P53 is an important immunohistochemical marker for early diagnosis and grading of Urothelial carcinoma cases.

***Keywords*:-** *P53, Urothelial Carcinoma, Immunohistochemistry.*

I. INTRODUCTION

Urothelial carcinoma (UC) is a common malignancy of the genitourinary tract with more than half a million new cases, and a mortality of almost two hundred thousand globally, in 2018. Histologically, UC is said to be the most common urinary bladder tumor that comprises of more than 90% of all cases.[1] Bladder carcinoma is the 9th most common cancer worldwide.[2] In United States, it represents 4th most common cancer in males and 10th most common tumor in females with a male to female ratio of 3:1 and median age of diagnosis at 68 years.[3] In Pakistan, a study conducted in Armed Forces Institute of Pathology, Rawalpindi showed that UC is the7th most commonly occurring tumor in both men and women and represents 93.4% of all bladder malignancies.[2] Smoking is the one of

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most significant risk factors which has strong association with bladder cancer.[4] Other factors include cyclophosphamide, phenacetin, auramine, some dyes, Schistosoma and previous irradiation of urinary bladder for treatment of prostatic carcinoma.[5] WHO/ISUP, in its latest classification of tumors of urinary tract, classified Urothelial tumors into invasive and non-invasive Urothelial neoplasms. Among the invasive UCs, various subtypes like Nested, Microcystic, Sarcomatoid, Micropapillary, Plasmacytoid, Giant cell, Lymphoepithelioma like, Clear cell and poorly differentiated, are included. The non-invasive Urothelial neoplasms are subdivided into UC in situ, papilloma, papillary urothelial neoplasm of low-grade malignant potential (PUNLMP), low grade Urothelial carcinoma (LGUC), and high-grade Urothelial carcinoma (HGUC).[6] Prognosis of UC is determined by various factors like pathological tumor grade and stage, muscularis propria invasion and patient's age.[7] Among these, grade of the tumor is the single most significant prognostic factor. The prognosis of LGUC is generally good, though these tumors show high recurrence rates. Approximately 30% of these recurrent tumors progress by invading lamina propria.[8] Genetics play a very crucial role in causation and progression of UC. P53 is a tumor suppressor gene on chromosome 17p, which is a frequently mutated gene observed in carcinoma of lung, breast and urinary bladder.[9] Wild-type p53 aids to inhibit tumor proliferation by averting the neovascularization carried by production of endogenous vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF). Mutated p53 loses its crucial regulatory role and hence, there is unchecked neovascularization, promoting tumor multiplication and advancement to progress.[10] Expression of p53 has both diagnostic as well as prognostic importance in Urothelial tumors. Over expression of p53 occurs in high proportion of Urothelial tumors, particularly high-grade forms and correlates well with prognosis. It is also an indicator of p53 mutation in neoplastic cells.[9]This study was intended to establish diagnostic utility of p53 antibody in classifying UC into high grade and low grade forms based on expression of p53. This classification will help in predicting prognosis and outcome of therapy, since LGUC carry better prognosis and 5-year survival rates as compared to HGUC.

II. METHODOLOGY

A retrospective cross-sectional study was carried out at the Histopathology department, Lady Reading Hospital (Medical Teaching Institution), Peshawar, from August 2021 till February 2022. Using WHO sample size calculator, the sample size calculation was done, keeping into account these parameters; Confidence level (1-a=95 %Anticipated population proportion (P) = 49.5%Absolute precision required (d) = 10 %Minimum sample size (n) = 97). Formalin fixed, paraffin embedded (FFPE), blocks of 97 cases UC were included in the study using non-probability, consecutive sampling technique. The biopsies which were inadequate, autolyzed or showing preservation and fixation artifacts were excluded from the study. Proforma of the patients for data collection was filled with record retrieved from data bank of Pathology department and Health management information system (HMIS) of the institution. Retrieved blocks were cut at 3-5um thickness for routine H&E staining and p53 immunohistochemical staining. DAKO kit was used for immunohistochemistry according to guidelines of manufacturer. Non neoplastic urinary bladder tissue was used as negative control while FFPE block of skin tissue was used as positive control. Slides were examined by two consultant Histopathologist, blind to the original issued reports. Histopathologic diagnoses were made for all the cases included in study and recorded in a predesigned proforma. P53 antibody stained slides were than evaluated. Each case was assigned positive or negative status, based on nuclear staining intensity and percentage score. Strong nuclear immunoreactivity in greater than 10 % tumor cells was regarded positive. Negative status was recorded for a case with either no staining at all or staining in fewer than 10% of tumor cells. Variables like age, gender, grade of tumor, muscle invasion and p53 status were recorded. For the purpose of statistical analysis, Spearman correlation was employed. Calculation of mean and standard deviation was performed for numerical variables like age. Tumor grade, gender, age and p53 immunohistochemical expression were expressed in the form of percentages and frequencies. Effect modifiers including age, tumor grade and gender were controlled by stratification. Spearman correlation was applied and p-value of less than 0.05 was considered significant.

III. RESULTS

Among 97 cases, the minimum and maximum age of patient recorded was 20 years and 90 years (as shown in Figure 1) with mean± standard deviation as 64± 11 years respectively. There were 86 (88.6%) male patients and 11 (11.3%) female patients which makes male to female ratio of 7.8:1. Among male patients, 42 cases of HGUC and 44 cases of LGUC were diagnosed. Among the 11 female patients, 06 cases showed high grade morphology while 05 cases were classified as LGUC. Among 97 cases, LGUC was seen in 49 (51%) while sections from 48(49%) cases showed high grade features. Immunohistochemically p53 was found positive in 63(65%) patients and it was negative in 34 (35%) patients as shown in Table 1. Among the positive cases, 45 were HGUC and 18 were LGUC. Out of 34 negative cases, 03 cases showed high grade morphology while 31 cases showed features of LGUC as shown in Table 1.

Table 1 Expression of p53 in UC

Tumor Grade	P53 Expression				P-Value
	Negative	Frequency (%)	Positive	Frequency (%)	
High Grade	03	3.09	45	46.39	p<0.05 *
Low Grade	31	31.96	18	18.56	
Total	34	35.05	63	64.95	

*P-Value is Significant at <0.05 Level.

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Table 2 Correlation of P53 Expression and Muscle Invasion

P53 Expression	Muscle Invasion		P-Value
	Present	Absent	
Negative	04	30	p<0.05*
Positive	54	9	
Total	**58**	**39**	

*P-Value is Significant At <0.05 Level.

Among the p53 positive cases, 54 cases showed muscle invasion while 04 cases of muscle invasive UC did not show p53 expression making significant correlation between muscle invasion and p53 expression (p-value <0.05) as shown in Table 2.

Table 3 Correlation of P53 Expression with Tumor Grade

Tumor Grade	P53 Expression				
	Negative	Frequency (%)	Positive	Frequency (%)	P-Value
High Grade	03	6.25	45	93.75	p<0.05 *
Low Grade	31	63.3	18	36.7	
Total	**34**		**63**		

*P-Value is Significant at <0.05 Level

Table 4 Correlation of P53 Expression and Patients' Gender

Gender	P53 Expression		P-Value
	Negative	Positive	
Male	27	59	P<0.05*
Female	7	4	
Total	**34**	**63**	

*P-Value is Significant at <0.05 Level.

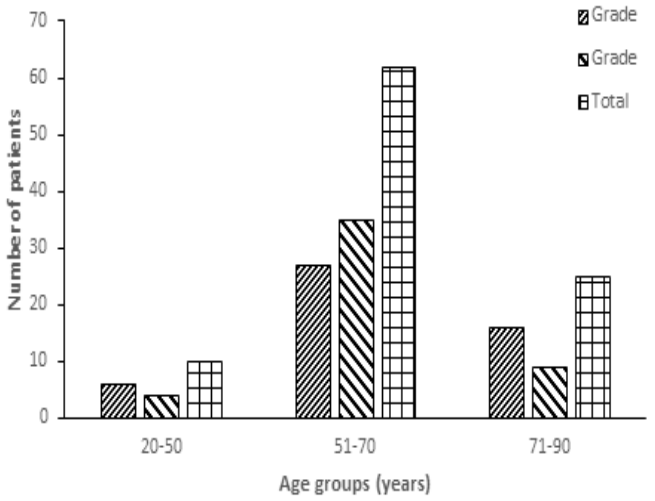

Fig 1 Distribution of Age Groups with Tumor Grade

Expression of p53 was seen in 59 male patients and 04 female patients while no expression was observed in 27 male patients and 7 female patients as demonstrated in Table 4. Among 48 cases of HGUC, 45 (93.75%) cases showed p53 positivity while 3(6.35%) cases did not show any p53 antibody staining. 18 (36.7%) cases of LGUC showed p53 positivity while 31(63.3%) cases (p-value <0.05) as shown in table 3.

IV. DISCUSSION

With the advancement in diagnostic methods over the last two decades, it is now common to use immunohistochemical markers for assessing predictive and prognostic potential of various tumors. Numerous immunohistochemical markers have been inspected in cases of UC of urinary bladder.[11] In the current study, we assessed the role of p53 in grading UC. P53 is a cancer suppressor gene which is strongly positive in high grade UCs. 97 patients were included in our study with calculated mean $\pm$ standard deviation for age as 64 $\pm$ 11 years and male to female ratio of 8:1. Another study showed male to female ratio of 4:1 with the same number of participants in their research.[5] Other investigators have published male to female ratio of 7.46:1, in cases of UC, which is concordant with our results.[12] We did not find any substantial association between age group and grading of UC. In Turkey, a study was conducted, over 18 years, to see the possible effect of age on the expected behavior and progression of bladder tumor in different age groups. They found that single and small tumors were usually present in patients younger than 40 years with lower recurrence rate but with similar tumor progression rate in both young and old age groups. They concluded their manuscript with remarks that invasiveness of tumors in young patients should be cautiously evaluated, and earlier intervention should be initiated for halting the progression for better outcome.[13] A multicenter research conducted in Iraq in 2018, stated that expression of p53 and p21 was strongest in high grade and muscle invasive UC.[14] These results are concordant with our findings since 54/58 (93.1%) cases of muscle invasive carcinoma of both low grade and high grade types displayed positive p53 staining as shown in Table 2. The product of altered p53 gene accumulates in tumor cells nuclei and is detected by immunohistochemistry.[15] In the current project, 63 (65%) of the cases yielded positive results while 34(35%) cases showed negative results for p53 overexpression on application of p53 antibody. Among p53 positive cases, 45 cases were HGUC and 3 cases were LGUC. Among the negative 34 cases, 31 cases were LGUC while 3 cases showed high grade morphology on H&E stained sections. Negative expression of p53 in these cases can be delineated by the fact that in spite of p53 gene mutation, protein product does not gather in the nucleus of 15% to 20% of tumors.[16] This is because point mutations in p53 result in absence of or severe reduction in synthesis of p53 protein. Some tumors show nuclear accumulation of p53 protein product, in the absence of gene mutation. In such cases, it has been proved that accumulation of some gene products like MDM2 deactivate wild-type p53 protein, consequently resulting in a prolonged half-life of *p53* gene products. MDM2 overexpression causing overexpression of *p53* with no *p53* gene mutation is elaborated by a research carried out by Özyalvacli G et al.[16] Nonetheless, *p53* expression was statistically substantial in high grade urothelial cancers in present study and the same is indicated by results of various other research projects.[15,16,17]

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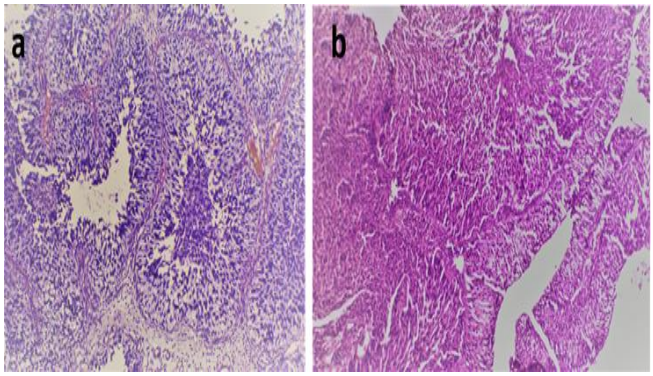

Fig 1 Papillary UC (a) High Grade; H&E, X 200 (b) Low Grade; H&E, X 200

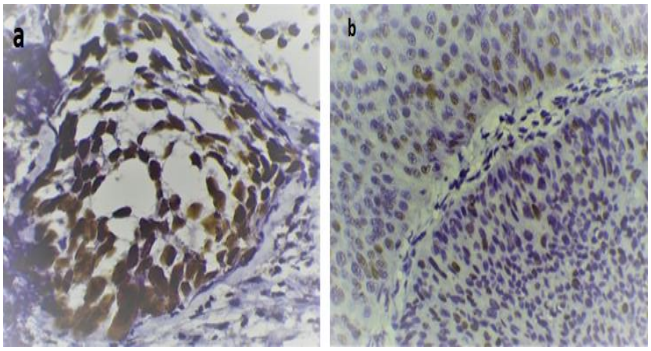

Fig 2 Papillary UC (a) High Grade; Strong P53 Positivity, X 400 (b) Low Grade; Weak P53 Positivity, X 200

Overexpression of p53 gene in UC has also been investigated by Yin H et al. They applied CK20, Ki67 and p53 on 84 cases of noninvasive papillary Urothelial neoplasms. According to their results, all benign neoplasms showed negative immunostaining for p53 antibody, with a significant difference between high and low-grade UCs. Only 21% LGUC included in their study expressed p53 antibody.[17] These findings are in accordance with our study since 18% of p53 negative cases and 02% of p53 positive cases showed low grade morphologic features. Our results are compatible with another experiment carried out by Mumtaz et al.[5] 73% of their cases with High grade features and 36% cases with low grade morphology showed p53 protein overexpression.[5] In a study carried out at King Edward Medical University, Lahore, from January to December 2016, p53 was positive in 91% of HGUC and 16% of LGUC.[9] Our findings are similar to this study, since p53 positivity was seen in majority of HGUC included in our project. P53 staining in non-muscle invasive UC is also investigated by R. Stec *et al* who showed that 96.27% of their cases stained positively for p53 protein.[18] The results of a trial conducted by Stadler et al. suggested that there is positive correlation between p53 gene mutation and grade of tumor. They showed that p53 positivity was higher in grade 3 and grade 4 UCs.[19]These conclusions are in harmony with our results. The results of a trial conducted by Stadler et al. suggested that there is positive correlation between *p53* gene mutation and grade of tumor. They showed that p53 positivity was higher in grade 3 and grade 4 UCs.[19] These conclusions are in harmony with our results. The prognostic value of p53 in UC is outlined by many researchers in recent literature.[20,21,22] Our findings are similar to all these studies. In a trial conducted at Egypt, it was reported that increased p53 was seen mainly in high grade tumors as compared to low grade tumors.[23] P53 positivity in our study was similar to this trial, since more cases of HGUC stained positive as compared to LGUC. Roy Chowdhury and colleagues demonstrated nuclear p53 positivity in 90% of low grade and 100% of high grade tumors, however they concluded their research by stating that p53 gene might be unrelated to development of urothelial neoplasm, since cases of PUNLMP show negative staining for p53 antibody.[24] The present findings are in contrast to that. In the present study, intense p53 positivity was noted in cases of HGUC, weak staining in cases of LGUC and no staining in a High-grade tumor that showed rhabdoid differentiation. This may suggest that when Urothelial papillary tumor dedifferentiates, it accumulates mutations other than p53. A project carried out by He *et al.* emphasized upon RAS pathway activation and prognostic role of RAS in UC that are p53 deficient.[25] These findings were confounded by Zhou and colleagues, who outlined the role Fibroblast Growth Factor3 Beta (FGR3b) in cell proliferation and tumor progression.[26] In our project, 3 cases of HGUC and 31 cases of LGUC were negative for p53 antibody. This negativity might be due to mutations, other than p53, involved in initiation and progression of these tumors. Positivity of p53 correlated well with higher grade and stage of UC.

V. CONCLUSION

UC progresses by acquiring mutations, notably mutations in p53. This mutation has a diagnostic and prognostic value. Immunohistochemical staining for p53 antibody not just aid in early diagnosis but it also underscores important prognostic connotation. Strong p53 positivity is seen in cases of HGUC while weak to absent staining is seen in cases of LGUC. Moreover, p53 negativity is also required in patients with UC undergoing treatment with bacille Calmette–Guerin. We recommend using p53 antibody in cases of UC since it has a diagnostic and prognostic value.

- *Conflict of Interest*
  The authors do not have any conflict of interest.

- *Acknowledgement:*
  This work has not been presented at any conference/symposium.

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