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Study of p53 Gene Expression in Urinary Bladder Carcinoma: Prospective & Retrospective Study

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Abstract

Introduction: The present study of Urothelial carcinoma is intended to include: Retrospective hospital based observational study of paraffin section of old block and cases attending our hospital. *Material Methods*: The study is conducted in the Department of Pathology at SRMS IMS, during a period of 21 months, 1st January 2013- 30th September 2014. Total 80 cases of Urothelial Carcinoma were included in the study. Tumour is classified according to WHO classification and all the 80 cases were subjected to Immunohistochemical study for p53 from the representative areas. Samples demonstrating at least 10% nuclear reactivity were considered positive for p53. *Results*: Out of 4 cases of PUNLMP, 1 case (25%) was positive for p53. Out of 27 cases of Low grade papillary urothelial carcinoma, 4 cases (14.8%) were positive for p53. Out of 48 cases of High grade papillary urothelial carcinoma, 28 cases (58.3%) were positive for p53. 1 case of Mucinous adenocarcinoma with signet ring change was positive for p53. *Discussion*: The present study results were in consistency with the other studies, i.e a higher rate of p53 protein positive staining was seen in high grade tumor. *Conclusions:* p53 alterations are significantly associated to clinicopathological features of poor prognosis, the inclusion of p53 mutation status into a predictive panel of tumor markers for bladder cancer is recommended.

Keywords: p53; Urinary Bladder Carcinoma; IHC.

Introduction

Bladder cancer is the ninth most common cancer in the world, with 430,000 new cases diagnosed in 2012 [1]. In the United States, almost 75,000 new cases and 16,000 deaths occur each year due to bladder cancer [2].

It is considerably more common in males than in females (ratio worldwide is about 3.5:1) [3]. In both sexes, the highest incidence rates of bladder cancer are observed in Western Europe, North America & Australia [4].

The TP53 gene, located at 17q23 encodes a 53kDa protein which plays a role in several cellular processes including cell cycle, response to DNA damage, cell death & neovascularization [5].

Mutations of TP53 gene, mostly located in the central, DNA binding portion of the gene, are a hallmark of invasively growing bladder cancers. An online query of the International Agency for Research on Cancer (IARC) database (R7 version, September 2002) revealed TP53 mutations in 40-60% of invasive bladder cancers (in studies investigating at least 30 tumors) [6,7].

Often TP53 mutations can be detected immunohistochemically since many TP53 mutations lead to protein stabilization resulting in nuclear TP53 accumulation. In addition to a postulated role as a prognostic marker, immunohistochemical TP53 positivity is a strong argument for the presence of genetically instable neoplasia in cases with unquestionable morphology. A recent metaanalysis of more than 3700 tumors found a weak but significant association between TP53 positivity & poor prognosis [8].

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Material and Method

The present study of Urothelial carcinoma is intended to include: Retrospective hospital based observational study of paraffin section of old block and cases attending the hospital.

The present study consist of 80 cases of urothelial tumors from the histopathology records in the department of Pathology, U.P for the period of 21 months i.e. from 1st January 2013- 30th September 2014. Specimen were sent from the department of Urology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly. The specimens were received in the form of Transurethral resection of bladder tumor chips. All the specimens obtained were fixed in buffered 10% formalin. Fixation time was 12 -24 hours, and then processed.For light microscopy one slide from each block was routinely stained with H & E.

After histopathological diagnosis of Urothelial carcinoma, tumour is classified according to WHO classification and all the 80 cases are subjected to Immunohistochemical study for p53 from the representative areas of the tumour. The p53 Protein, Monoclonal (D07), Mouse, IgG2b based IHC kit of BioGenex RTU was used.

Results and Observations

The present study was carried out in the department of Pathology, SRMS Institute of Medical sciences, Bareilly. Attempts are made to correlate the presence of p53 mutation in tumors of urinary bladder.

A total number of 80 cases of urothelial tumors were analysed.

1. Most of the cases were Hindus with a Hindu-Muslim ratio of 5.2:1.
2. Majority of the cases of urothelial tumors fall in the 6th decade comprising of 30 cases (37.5%).
3. Males were predominantly affected with 72 cases (90%).
4. Majority of the patients presented with painless hematuria as chief complaints with 64 cases (80%).
5. Mean age of presentation was 58yrs.
6. Minimum age of presentation was 21yrs who presented with Low grade papillary urothelial carcinoma. Maximum age of presentation was 82yrs who had High Grade Urothelial Carcinoma (Lamina invasive).
7. According to WHO/ISUP classification, out of 80 cases, 48 cases (60%) were reported as High Grade papillary Urothelial Carcinoma, 27 cases (33.75%) were of Low Grade Papillary Urothelial Carcinoma, and 4 cases (5%) were of PUNLMP, 1 case (1.25%) was of Mucinous adenocarcinoma.
8. According to histological typing on the basis of invasion, out of 76 cases, maximum 32 cases (42.1%) were reported as High Grade papillary urothelial carcinoma with muscularis propria invasion; 12 cases (15.7%) as High Grade papillary urothelial carcinoma with only lamina propria invasion, and 4 cases (5.26%) of High Grade papillary urothelial carcinoma did not show any invasion.
9. In Low grade papillary urothelial carcinoma, only 2 cases (2.6%) showed muscularis propria invasion, and 9 cases (11.8%) showed only lamina propria invasion, while 16 cases (21.05%) did not show any invasion.
10. Maximum number of cases of PUNLMP, Low grade papillary urothelial carcinoma, High Grade Papillary Urothelial Carcinoma were reported in the age group of 51-60 years.
11. p53 immunostaining showed a positive result in 42.5% cases of all Urothelial tumors.
12. p53 immunostaining showed positive result in 58.3% of cases of High papillary urothelial carcinoma,14.8% of Low Grade papillary urothelial carcinoma. Hence not all cases of urothelial carcinoma show p53 expression suggesting that, though p53 plays a pivotal role in carcinogenesis, there must be many other co factors involved as well.
13. p53 immunostaining showed positive result in 60.7% cases of High Grade papillary urothelial

Table 1: Distribution of variants of high grade urothelial carcinoma

S. No.	Diagnosis	No. of Cases	Percentage
1	High grade papillary urothelial carcinoma	35	72.9%
2	Mixed high grade papillary urothelial carcinoma and adenocarcinoma	1	2.1%
3	High grade urothelial carcinoma with spindle cell differentiation	2	4.2%
4	High grade urothelial carcinoma with squamous differentiation	6	12.5%
5	High grade urothelial carcinoma with glandular differentiation	4	8.3%
	Total	48	100%

Table 2: Distribution of cases on the basis of invasion

S. No.	Diagnosis	No. of cases	Percentage
1	Low grade papillary urothelial carcinoma without invasion	16	21.06%
2	Low grade papillary urothelial carcinoma with only lamina propria invasion	9	11.8%
3	Low grade papillary urothelial carcinoma with muscularis propria invasion	2	2.6%
4	High grade papillary urothelial carcinoma without invasion	4	5.26%
5	High grade papillary urothelial carcinoma with only lamina propria invasion	12	15.7%
6	High grade papillary urothelial carcinoma with muscularis propria invasion	32	42.1%
7	Mucinous adenocarcinoma with signet ring change with muscularis propria invasion	1	1.3%
	Total	76	100%

Table 3: Distribution of p53 positivity in variants of high grade urothlial carcinoma

S. No.	Diagnosis	No. of cases	No. of cases positive for p53	Percentage
1	High grade papillary urothelial carcinoma	35	22	62.8%
2	Mixed high grade papillary urothelial carcinoma and adenocarcinoma	1	1	100%
3	High grade urothelial carcinoma with spindle cell differentiation	2	1	50%
4	High grade urothelial carcinoma with squamous differentiation	6	2	33.3%
5	High grade urothelial carcinoma with glandular differentiation	4	2	50%
	Total	48	28	58.3%

Table 4: Comparison of p53 positivity with other studies

Study	Year	Site	No. of Cases	% OF p53 +VE Cases
Current Study	2015	Bareilly, UP	80	42.5%
Venyo A. et al.[35]	2010	U.K.	86	54%
Turk N. et al.[36]	2009	Turkey	84	47.6%
Ibrahim N. et al.[22]	2009	Egypt	74	74.3%
El-chennawi F.et al.[23]	2009	Egypt	50	66%
Abdul- Hameed A.et al[37]	2007	Karbala-Iraq	58	50%
Galmozzi F. et al.[38]	2006	Italy	82	73.17%
Comperat E. et al.[39]	2005	France	158	63.9%
Serdar A. et al.[40]	2005	Turkey	61	64%
Cheng H.et al.[12]	2001	China	142	19%
Barsoum H. et al.[24]	2000	Egypt	49	67.3%
Shiina H. et al.[13]	1999	Japan	84	20.5%
Okamura T. et al.[25]	1998	Japan	79	42%

Table 5: Comparison of percentage of cases according to various age groups with another study

Age (year)	Current Study		Tawfeeq et al. [21]	
	Total	%	Total	%
≤50	23	28.75%	8	16.00%
51-60	30	37.50%	11	22.00%
61-70	17	21.25%	21	42.00%
>70	10	12.50%	10	20.00%
Total	80	100%	50	100%

Table 6: Comparison of percentage of p53 positivity in various age groups with another study

Age (year)	Current Study		Tawfeeq et al.[21]	
	No. of cases positive for p53	%	No. of cases positive for p53	%
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51-60	30	37.50%	11	22.00%
61-70	17	21.25%	21	42.00%
>70	10	12.50%	10	20.00%
Total	80	**100%**	50	**100%**

Table 7: Distribution of variants of high grade urothelial carcinoma

S. No.	Diagnosis	No. of Cases	Percentage
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5	High grade urothelial carcinoma with glandular differentiation	4	8.3%
	Total	48	100%

Table 8: Distribution of cases on the basis of invasion

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7	Mucinous adenocarcinoma with signet ring change with muscularis propria invasion	1	1.3%
	Total	76	100%

Table 9: Distribution of p53 positivity in variants of high grade urothlial carcinoma

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5	High grade urothelial carcinoma with glandular differentiation	4	2	50%
	Total	48	28	58.3%

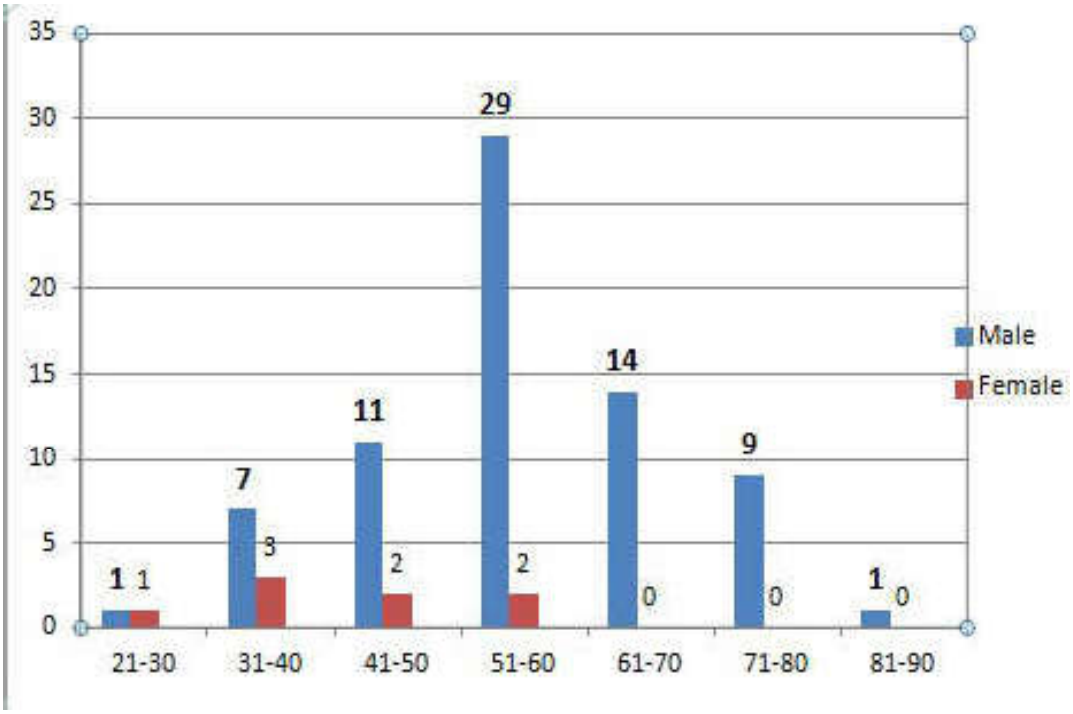

Fig. 1: Gender wise distribution of cases

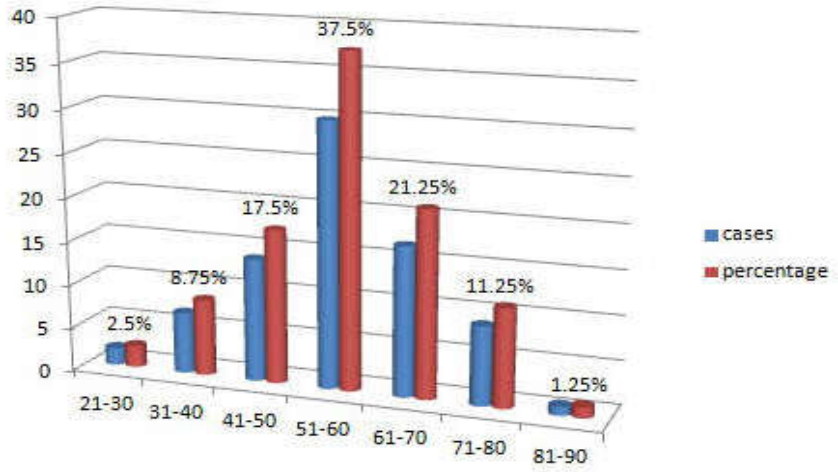

Fig. 2: Age wise distribution of cases

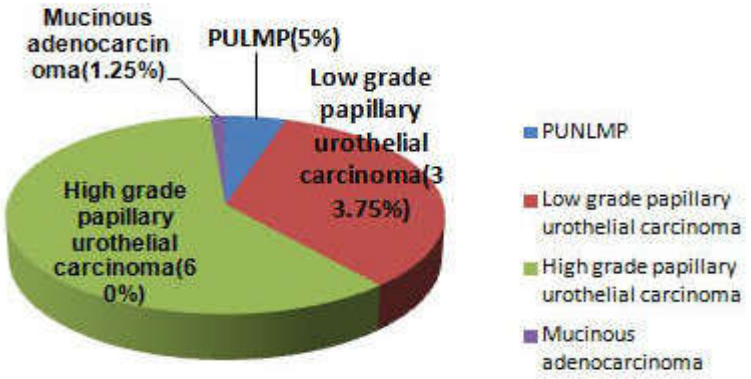

Fig. 3: Distribution of cases according to histopathological diagnosis

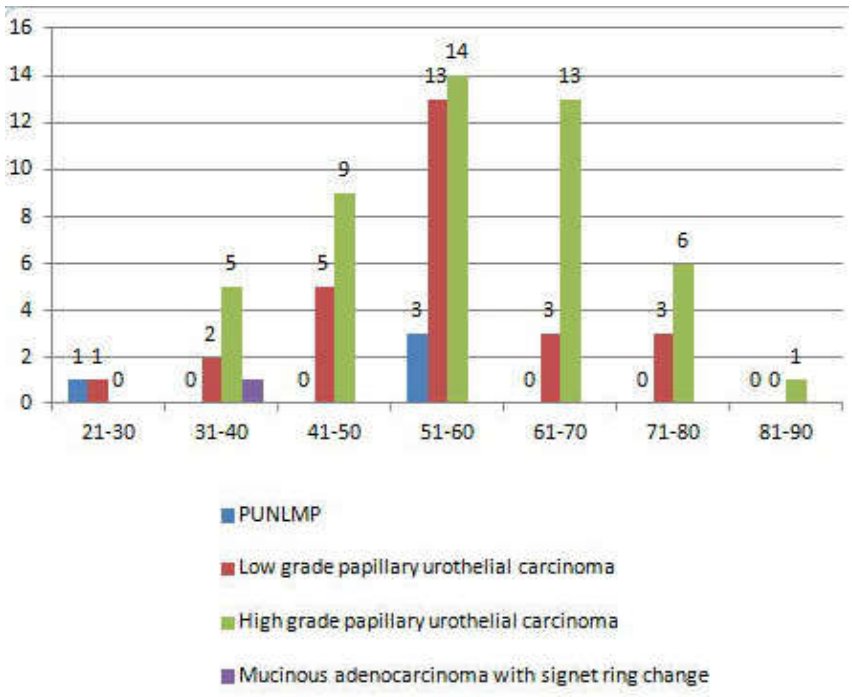

Fig. 4: Distribution of cases according to age group

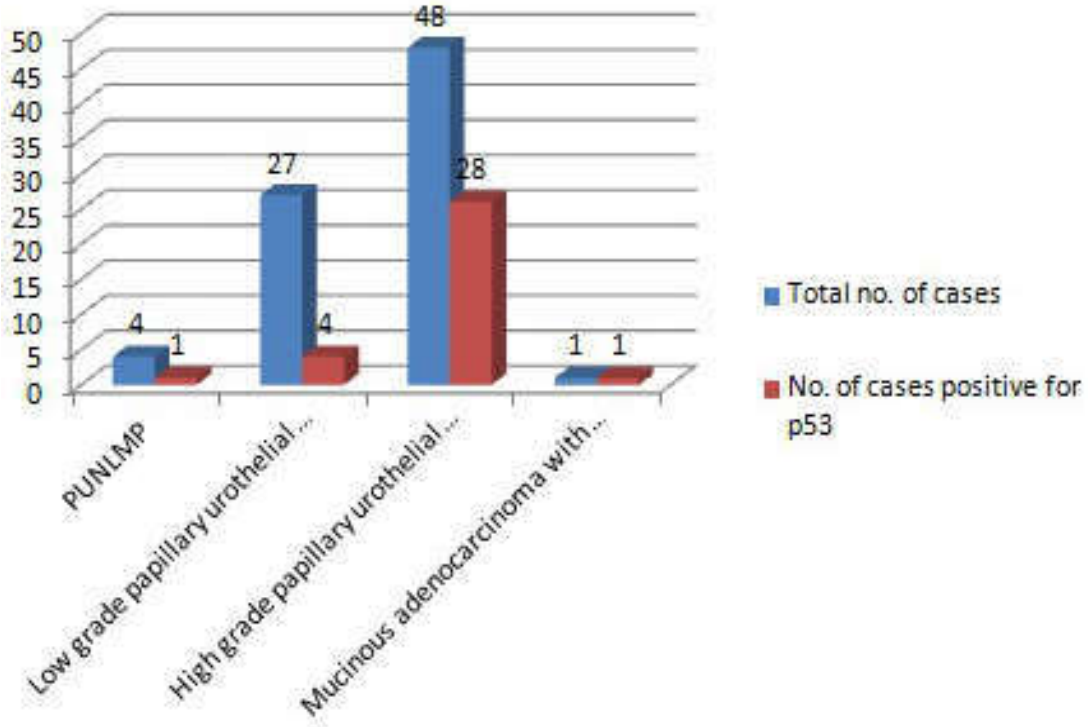

Fig. 5: Percentage of p53 positivity in various types of urothelial tumors

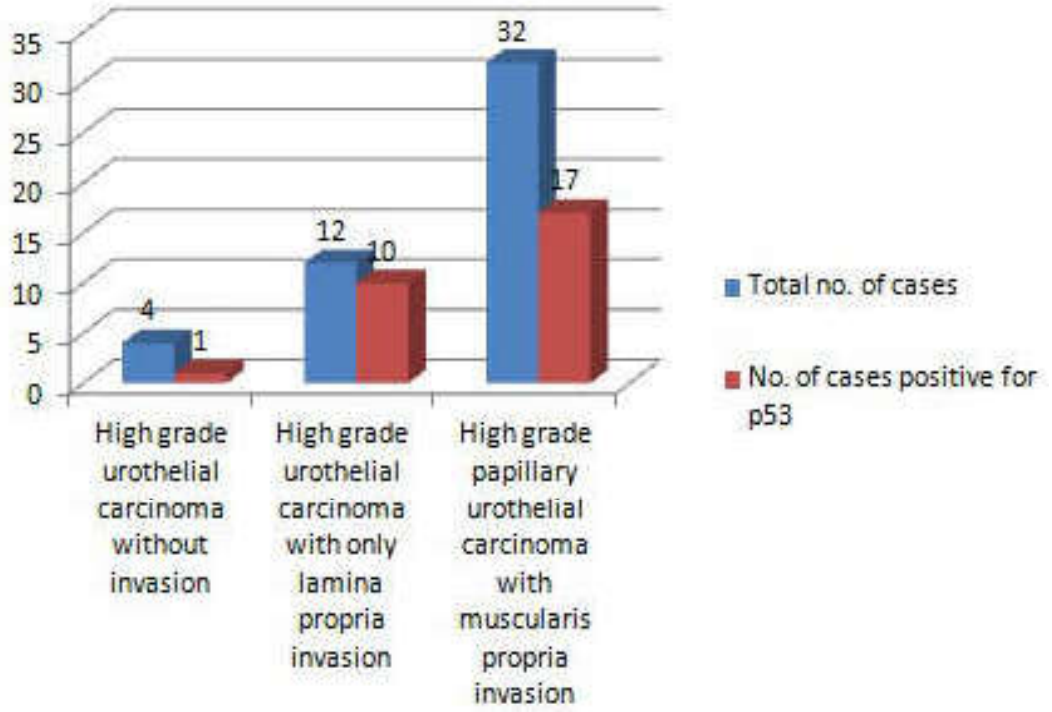

Fig. 6: Percentage of p53 positivity in various types of high grade urothelial carcinoma on the basis of invision

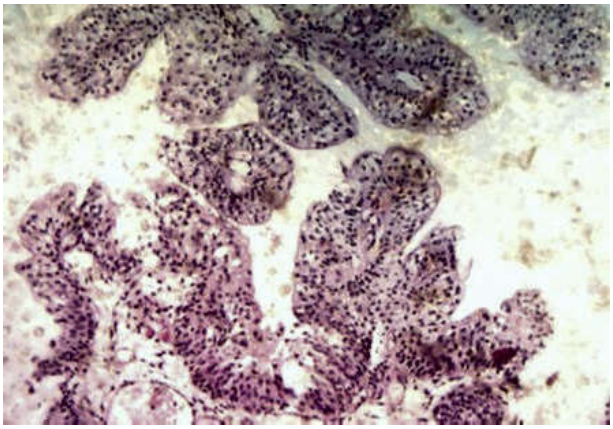

Fig. 7: PUNLMP (H & E: 10X)

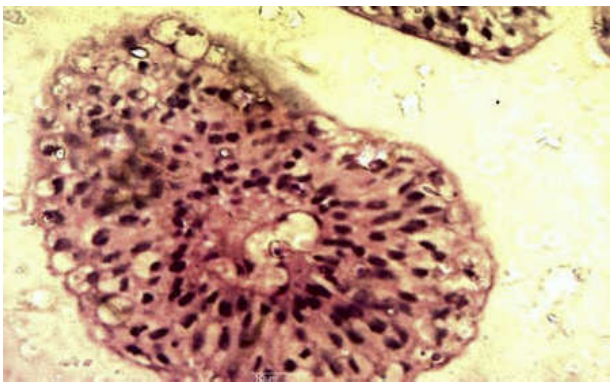

Fig. 8: PUNLMP (H & E: 40X)

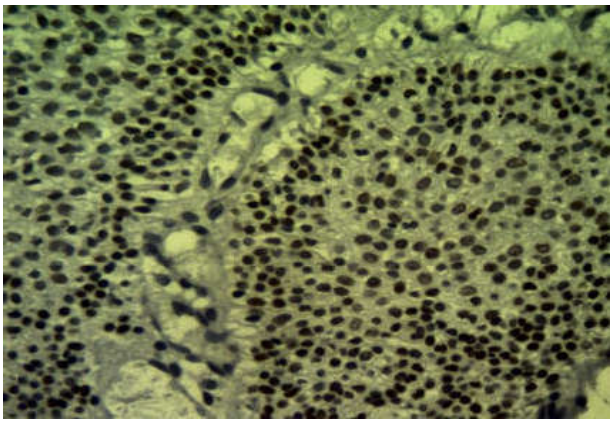

Fig. 9: p53 positive PUNLMP (40X)

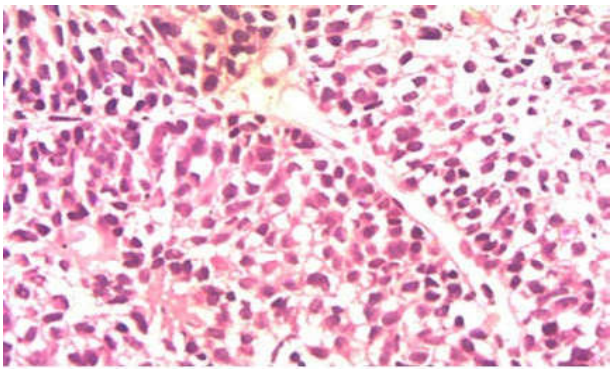

Fig. 10: Low grade papillary urothelial carcinoma (H & E:40X)

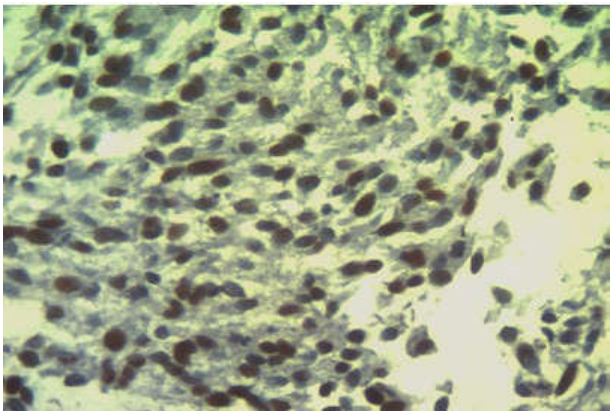

Fig. 11: p53 positive low grade papillary urothelial carcinoma (40X)

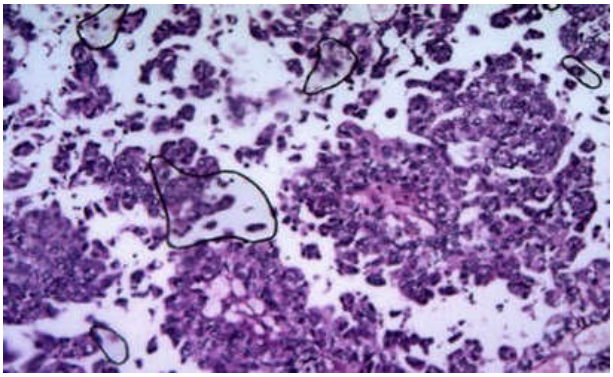

Fig. 12: High grade papillary urothelial carcinoma (H&E: 10X)

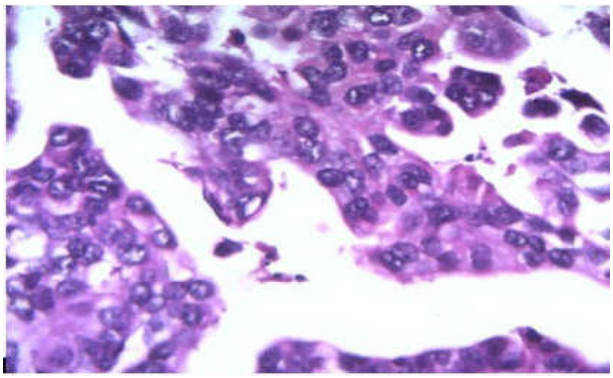

Fig. 13: High grade papillary urothelial carcinoma (H&E:40X)

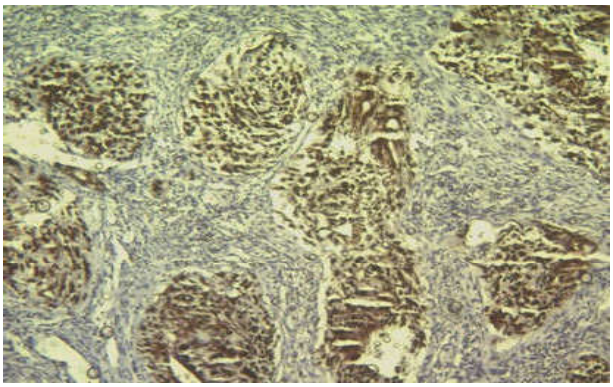

Fig. 14: p53 positivity seen in high grade urothelial carcinoma (10X view)

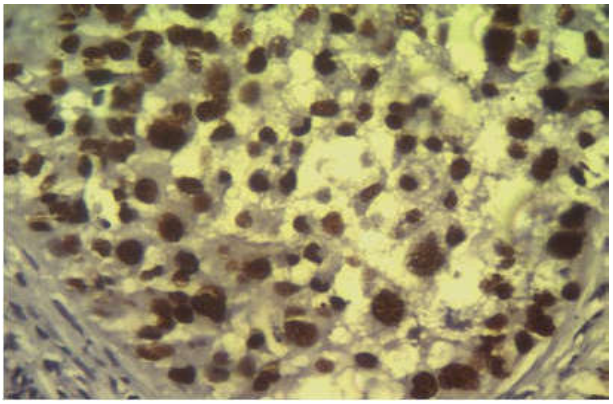

Fig. 15: p53 positive high grade papillary urothelial carcinoma (40X view)

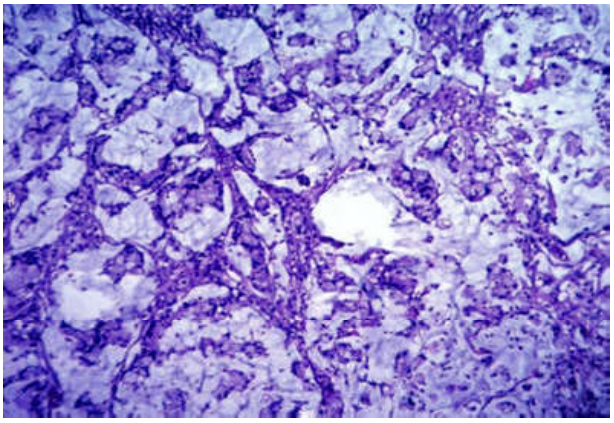

Fig. 16: Mucinous adenocarcinoma with signet ring change (H&E: 10X)

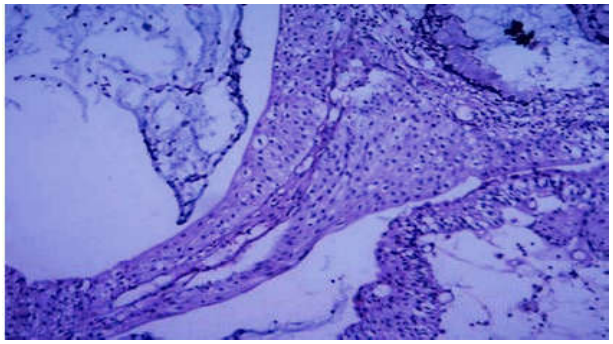

Fig. 17: Mucinous adenocarcinoma with signet ring change (H&E: 40X)

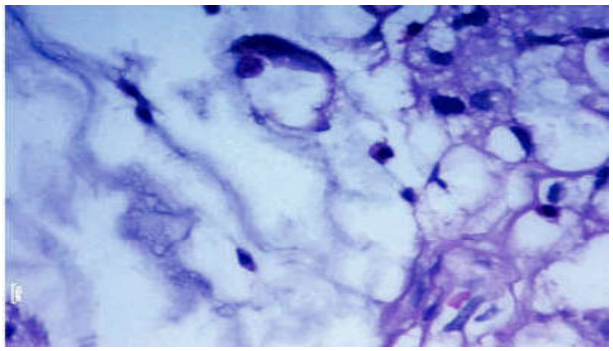

Fig. 18: Signet cell seen in a case of mucinous adenocarcinoma (H&E: 40X)

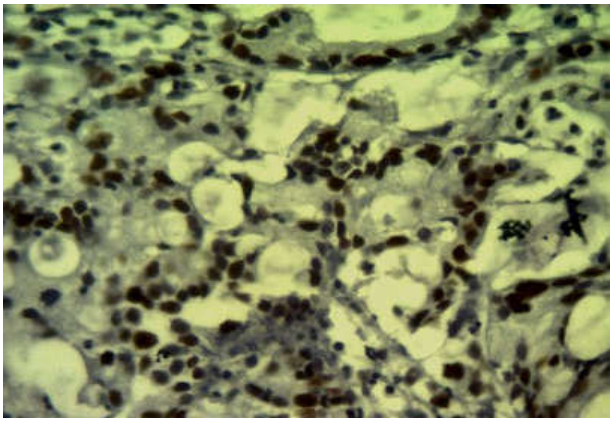

Fig. 19: p53 positive mucinous adenoccarcinoma (40X)

carcinoma with muscularis propria invasion, 35.7% cases of High Grade papillary urothelial carcinoma with only lamina propria invasion, and 3.6% of High grade papillary urothelial carcinoma without invasion.

Discussion

The over-expression of p53 was nuclear in all tumor tissues, which confirms previous observations [9].

There was no correlation between p53 over-expression and any of the clinicopathologic characteristics recorded. Unfortunately, published data on p53 in bladder carcinomas are not sufficient to enable direct comparisons [10].

In the current study, 42.5% of patients with Urothelial tumors showed p53 over expression. Although the sample size is small, similar results had been observed by others [11-13], Sink Z. et al, 1996, studied 44 patients with TCC and showed that p53 was over expressed in 18.2% [11].

In Iraq, Al-Qaysi, 2002, showed that p53 over expressed in 23 out of 40 bladder cancer patients (57.5%) [14]. In the other study, also conducted in Iraq, by Abdul-Hameed A. et al, 2007, showed that p53 over-expressed in 29/58 bladder cancer patients (50%) [15]. Other similar studies have shown variable ratios (18.2%-74.3%) (Table 4).

p53 Expression in Relation to Age

In the present study, the patient's age was in the range of 21 to 82 years with a mean of 58 years. In Turkey, by Emrbagci A, Yagci F, et al [16] the mean age of 63 years and age range of 34-87 years was seen. In Jordan, Matalka I, Bani-Hani K , et al [17] also found a comparable result to this study with a mean age 60.6

years and age range 19-91 years . In USA, Cheng L, Neumann R, et al [18] recorded a mean age of 69 years with age interval of 36-96 years. In another study in USA, Boudreaux K, Clark P, et al [19] found a mean age with 65.3 years and 35-84 years as age range. In Japan, Oh B, Sim J, Park CH,et al [20] study showed 61.7 years as mean age.

Regarding gender, in the current study the male to female ratio was (9:1). Other studies showed different values of male to female ratio for example Cheng L, Neumann R, et al [18], showed (4:1), Emrbagci A, Yagci F, et al [16] showed (5.4:1), Boudreaux K, Clark P, et al [19]showed (1.38:1) and Matalka I, Bani-Hani K, [17] showed (10:1).

p53 Expression and Age of Patient

In our study maximum no. of cases, 30 cases (37.50%) was seen in age group, 51-60 yrs, and minimum no. of cases, 10 (12.50%) was seen >70yrs age group. While Tawfeeq et al, at University of Mosul, [21] did their study in 50 patients. They observed maximum no. of cases, 21 cases (42.00%) in age group, 61-70, and minimum no. of cases, 8(16.00%) was seen in <50 years age group.

p53 over-expression was mainly found in the 7th decade (32.4%), Similar to study done by Tawfeeq et al,[21] (41.20%) Statistically, there was no significant correlation between p53 over-expression and the age of the patients. This is consistent with the conclusions of others [22-25].

p53 Expression in Relation to Grade of Tumors

Positive p53 staining was reported in 10-75% of low- grade tumors and in more than 58% of high grade tumors [26-31]. In a study by Ye et al, positive nuclear staining for p53 was shown in 34(51%) of 67 patients with Transitional cell carcinoma [32].Also Ye et al. have reported 40.8% and 78% p53 protein staining in low and high grade tumors, respectively [32]. Most of the previous studies have demonstrated that the rate of p53 expression in the patients with high grade tumor is higher than in patients with low grade tumor [27-31].In our study 1(25%) of 4 patients of PUNLMP, 4(14.8%) of 27 patients of Low grade tumor, 28(58.3%) of 48 patients of High grade tumor showed p53 protein staining respectively. Thus the present study results were in consistency with these studies, i.e a higher rate of p53 protein positive staining was seen in high grade tumor. Higher percentage of p53 positivity, i.e. 25% seen in PUNLMP can be explained as we had only four cases of PUNLMP in our study. p53 immunostaining showed positive result in 17 cases (60.7%) of cases of High papillary urothelial carcinoma with muscularis propria invasion, 10 cases (35.7%) of High Grade papillary urothelial carcinoma with only lamina propria invasion, and 1 case (3.6%) of High grade papillary urothelial carcinoma without invasion.

p53 protein is considered as a prognostic marker that might reflect the potent aggressive malignancy, and poor prognosis [33], and the results of the current study confirmed it by demonstrating a higher rate of p53-protein positive staining in high grade papillary urothelial carcinoma. Esrig and colleagues [34] found that extraordinarily large amounts of the p53 protein (mostly mutated forms) in the nuclear region of more than 10% of examined cells from resected bladder tumors indicate a bleak prognosis with respect to both recurrent disease and long term survival. Because of their findings, Esrig et al [34] recommend adjuvant therapy, including chemotherapy and radiotherapy, for patients found to have extraordinarily high concentrations of (mutant) p53 in resected bladder tissue.

Conclusion

To conclude, Urothelial carcinoma is the most common type of bladder carcinoma. Stromal invasion by urothelial carcinoma proceeds in two stages: invasion of the lamina propria and invasion of the muscle layer. Detection of the former is a difficult and somewhat subjective exercise. Conversely, detection of muscle invasion is of great consequence because of its influence on therapy and prognosis. High grade urothelial carcinoma has a higher degree of association with p53 gene mutation. The incidence of p53 mutation is fairly common in Urinary bladder carcinoma.

In our study maximum number of cases were noted in 5^{th} to 6^{th} decade with most patients being males about 90% and 80% patients presented with painless hematuria.Our results indicated 42.5% cases of urothelial carcinoma were positive for p53 and 57.5% cases were negative. In the present study maximum number of cases of High grade papillary urothelial carcinoma were positive for p53 (58.3%).

Data from these studies suggest that genetic assays are necessary for the optimal determination of TP53 alterations, mainly in tumors with a p53 negative phenotype, and especially in early stage tumors for which p53 status may assist in determining its progression to invasive disease. Since p53 alterations

are significantly associated to clinicopathological features of poor prognosis, the inclusion of both p53 phenotype and TP53 mutation status into a predictive panel of tumor markers for bladder cancer is recommended.

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