

## P53 over-expression in urothelial carcinoma of the bladder: An Immunohistochemical Study

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### **Absract**

**Objectives:** The aim of this study is, to evaluate the frequency of p53 over-expression in urothelial carcinoma of the bladder, to correlate the over-expression with age and sex of the patients, and grade and stage of the tumor, and to compare the results with those of others.

**Methods:** The study was performed on 50 bladder cancers. Samples were obtained in a prospective and retrospective fashions (case series study). The samples were collected from Al-Jumhuri Teaching Hospital, during a period of 8 months from July 2011 through February 2012.

**Results:** The patients' age was in the range of 23 to 91 years with a mean of 62.64 year. Most of them were in the seventh decade (42%). P53 immunoreactivity was observed in 34% of the total cases, it was positive in 7/11 of PUNLMP cases, 6/23 of low grade cases, and in 4/15 of high grade cases, and was negative in the only case of papilloma. It's positivity was observed in 7/14 of cases with Ta stage, 5/ 21 of T1 stage, in 4/ 11 of T2 stage, and in 1/ 2 of Tis stage. It was negative in the 2 cases of T4. Statistically p53 over-expression was not significantly related to the age and sex of the patients, and grade, and stage of the tumors. It was mainly found in the 7th decade of life (41.2%), in males (88.2%), in PUNLMP grade (41.2%), and in stage Ta (41.2%).

**Conclusions:** P53 was over-expressed in 34% of urothelial carcinomas of the bladder, predominantly in PUNLMP (41.2%), followed by low grade tumors (35.3%), and high grade tumors (23.5%), while it was negative in papilloma. Statistically, p53 over-expression was neither significantly correlated with the age and sex of the patients nor with grade, or stage of the tumors.

**Key words:** urothelial carcinoma, P53 expression.

### **Introduction**

Bladder cancer is the fourth most common cancer in males and ninth most common cancer in females, and there is a 15-fold variation in incidence rates internationally. Some of the differences in incidence among countries are due to differences in the reporting of low-grade

urinary bladder tumors; however, this does

not explain all of the variations. (1). The incidence of carcinoma of the bladder is higher in developed than in developing nations, and in urban than in rural dwellers. The male-to-female ratio is approximately 3: 1. About 80%

of patients are between the ages of 50 and 80 years. Bladder cancer, with rare exceptions, is not familial. Over 350 000 new cases are diagnosed worldwide. (2)

Since the p53 tumor suppressor gene has been found to be mutated in more than 50% of human cancers, it has attracted the interest of numerous researchers. (3) The gene encoding p53 mediates a major tumor suppression pathway that is frequently altered in human cancers. (4) The reported p53 mutation rate in bladder cancer was in the range of 30%-58%. (5, 6)

The aim of this study is, to evaluate the frequency of p53 over-expression in urothelial carcinoma of the bladder, to correlate p53 over-expression with age and sex of the patients, and grade and stage of the tumor, and to compare the results with those of others.

### **Patients & methods**

This study is based on samples prospectively and retrospectively collected from 50 patients with urothelial carcinoma of the bladder. Samples were collected from Al-Jumhuri Teaching Hospital. Expression of p53 protein by immunohistochemical staining was studied and compared in relation to patient's age, and sex, and grade and stage of the tumors.

Hematoxyline and eosin stained slides from formalin-fixed paraffin-embedded biopsy blocks were reevaluated to verify the presence of urothelial carcinoma of the bladder, and for tumor

grading and staging.

Urothelial tumors were classified **into:**

- Urothelial papilloma.
- Papillary urothelial neoplasm of low malignant potential.
- Papillary urothelial carcinoma, low grade.
- Papillary urothelial carcinoma, high grade.

P53 over-expression was assessed immunohistochemically on formalin-fixed paraffin-embedded tissues of the tumor, using mouse monoclonal antibody (clone DO-7), RTU (Code No. / M 7001) Isotype: IgG2b, kappa and permanent red Envision system K4004.

Positive and negative control slides were included in each run of staining. Positive control slides were prepared from adenocarcinoma of colon known to be positive for p53. While negative control slides were prepared from the same tissue block but incubated with tris buffered saline (TBS) instead of the primary antibody. Immunohistochemical staining interpretation: A semi-quantitative histochemical score was used to record results of p53 nuclear staining, this system takes into consideration both the percentage and intensity of stained cells. The intensity of staining was graded subjectively into four categories as

follows: 1- no positive staining; 2- Slight or weak staining; 3-moderate staining; 4- heavy or intense staining. The extent of cellular involvement was similarly divided into four classes: 1-(0%-24%) of the cells; 2- (25%-49%); 3-(50%-74%); 4-(75%-100%). To obtain overall values the two figures were multiplied and a positive result arbitrarily defined as a product of 5 or more. (7)

More than 1000 tumor cells, in multiple high power fields, have been counted for assessing the percentage. Also the average staining intensity was considered. The slides were checked more than one time to exclude any error.

**Statistical analysis:** The relationship between p53 over-expression and the clinico pathologic variables was analyzed by the chi-square test. The results were considered statistically significant if the p-value was  $\leq 0.05$ .

## **Results**

For a period of 8 months (from July 2011 through February 2012), immunohistochemical study to assess the over-expression of p53 protein in 50 specimens from patients with urothelial tumors of the bladder was performed.

The patients' age was in the range of 23 to 91 years with a mean of 62.64 year. Most of them were in the seventh decade (42%).

There were 45 males (90%) and 5 females (10%). Histologically, there were 1 case of papilloma (2%), 11 cases of PUNLMP (22%), 23 cases of low grade carcinoma (46%), and 15 cases of high grade carcinoma (30%). Also histologically there were 14 cases in stage Ta (28%), 21 cases in stage T1 (42%), 11 cases in stage T2 (22%), 2 cases in stage T4 (4%), and 2 cases in stage Tis (4%).

**P53 expression:** P53 immunoreactivity was observed in 17 cases (34%) of the total (figure1).

**P53 expression and patient's age:**

The p53 over-expression has no significant correlation to age with p-value = 0.107, (table 1).

**P53 expression and patient's sex:**

The p53 over-expression has no significant correlation to sex with p-value =0.765, (table 2).

**P53 expression and grade of the tumors:**

The p53 over-expression has no significant correlation to the grade of the tumors with p-value=0.152, (table 3).

**P53 expression and stage of the tumors:**

The p53 over-expression has no significant correlation to the stage of the tumors with p-value=0.426, (table 4).

## **Discussion**

Urothelial cancer is one of the most common cancers in developed countries and transitional cell carcinoma (TCC) of the bladder accounts for the 90%–95% of urothelial tract cancers. (8)

P53 is the most widely investigated molecular marker in bladder cancer. (9) The p53 gene, located on chromosome 17p, maintains genomic integrity in the face of cellular stress from DNA damage. (10, 11) Alteration of p53 gene is the most common molecular aberration found in human tumors to date.

Mutated p53 is dysfunctional and has a prolonged half-life resulting in the nuclear accumulation of the abnormal protein. Mutations in p53 gene are not the only reason for accumulation of this protein in cancer cells. Kobayashi and Tsukamoto established that p53 could be up regulated by genes, such as jun, myc and other cascade proteins in signal transduction. (12)

### **P53 expression:**

The over-expression of p53 was nuclear in all tumor tissues, which confirms previous observations. (13) The DO-7 monoclonal antibody that was used in our study recognizes a short, denaturation resistant, highly immunogenic epitope (segment) of the wild type (WT) and mutant type (MT)

p53 protein. (14)

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. (15)

In the current study, 34% of patients with TCC showed P53 over expression. Although the sample size is small, similar results had been observed by others, (14, 16, 17) Sink Z. et al, 1996, studied 44 patients with TCC and showed that P53 was over expressed in 18.2%. (14)

In Iraq, Al-Qaysi, 2002, showed that P53 over expressed in 23 out of 40 bladder cancer patients (57.5%).(18) Also in Iraq, Abdul-Hameed A. et al, 2007, showed that P53 over-expressed in 29/58 bladder cancer patients (50%). (19) Other similar studies have shown variable ratios (18.2%-74.3%) (Table 5).

Possible sources for this variation may be related: (20)

- 1-To differences in staining procedures.
- 2-To interpretation of staining pattern.
- 3-To the adopted cut off values.

### **P53 expression in relation to age:**

In the present study, the patients' age was in the range of 23 to 91 years with a mean of 62.64

year. In western countries, the median age is 65 years. <sup>(28)</sup>

P53 over-expression was mainly found in the seventh decade (41.2%). Statistically, there was no significant correlation between p53 over-expression and the age of the patients. This is consistent with the conclusions of others. <sup>(7, 20, 23, 24)</sup>

Because the median age at diagnosis was 69 years old for males and 71 years old for females, <sup>(29)</sup> one age-related reason for an increased risk of cancer may be accumulation of somatic mutations. <sup>(30)</sup>

#### **P53 expression in relation to sex:**

There were 45 males (90%) and 5 females (10%). The percentage of p53 immunoreactivity was 88.2% for males and 11.8% for females. This might be due to hormonal differences, or tobacco smoking which is a main risk factor for bladder cancer.

Statistically, there was no significant correlation between p53 over-expression and sex of the patients. This is comparable to those of other studies. <sup>(7, 20, 23, 24)</sup>

#### **P53 expression in relation to grade of tumors:**

P53 over-expression was mainly found in PUNLMP (41.2%), however,

over-expression of p53 in relation to grade was not significant statistically as shown by others. <sup>(7, 19, 20, 23, 25)</sup> Others ((Venyo A. et al, <sup>(21)</sup> Turk N. et al, <sup>(22)</sup> El-chennawi F. et al, <sup>(24)</sup> Serdar A. et al, <sup>(27)</sup>) reported significant correlation with higher grades.

These contradictory results might be due to the presence of inter- and intra-individual variations in evaluation of tumor grades in patients with bladder tumors. <sup>(31, 32)</sup>

P53 expression in relation to stage of tumors:

The critical importance of tumor histological stage had been recognized in several studies. <sup>(8, 33)</sup>

In this study P53 over-expression was mainly found in Ta stage (41.2%). Statistically, the over-expression of p53 in relation to stage was not significant confirming the observation by others. <sup>(7, 23, 24, 25)</sup> On the contrary, some studies ((Abdul-Hameed A. et al, <sup>(19)</sup> Barsoum H. et al, <sup>(20)</sup> Turk N. et al, <sup>(22)</sup> Serdar A. et al <sup>(27)</sup>)) had reported significant correlation with higher stages.

#### **Conclusions**

1. P53 over-expression was found in 34% of bladder urothelial tumors, and this result is within the range observed by others.

2. P53 over-expression in descending order was observed: in PUNLMP grade (41.2%), in low grade

carcinoma (35.3%), and in high grade carcinoma (23.5%).

3. As far as histological stage, P53 over-expression was in descending order; 41.2% in Ta stage, 29.4% in T1 stage, 23.5% in T2 stage, and 5.9% in Tis stage.

4. Age and sex of the patients, and grade, and stage of the tumors had no significant correlation with p53 over-expression.

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**Table1: P53 expression and age of patient**

Age (year)	Total		+ve		-ve		p-value
	No.	%	No.	%	No.	%	
≤50	8	16.0	3	17.6	5	15.2	0.107
51-60	11	22.0	1	5.9	10	30.3	
61-70	21	42.0	7	41.2	14	42.4	
>70	10	20.0	6	35.3	4	12.1	
Total	50	100.0	17	34.0	33	66.0	

**Table 2: P53 expression and sex of patient**

Sex	Total		P53 +ve		P53 -ve		p-value
	No.	%	No.	%	No.	%	
Male	45	90.0	15	88.2	30	90.9	0.765
Female	5	10.0	2	11.8	3	9.1	
Total	50	100.0	17	34.0	33	66.0	

**Table 3: P53 expression and grade**

Grade	Total		P53 +ve		P53 -ve		p-value
	No.	%	No.	%	No.	%	
papilloma	1	2.0	0	0.0	1	3.0	0.152
PUNLMP	11	22.0	7	41.2	4	12.1	
Low grade	23	46.0	6	35.3	17	51.5	
High grade	15	30.0	4	23.5	11	33.3	
Total	50	100.0	17	34.0	33	66.0	

**Table 4: P53 expression and stage**

Stage	Total		P53 +ve		P53 -ve		p-value
	No.	%	No.	%	No.	%	
Ta	14	28.0	7	41.2	7	21.2	0.426
T1	21	42.0	5	29.4	16	48.5	
T2	11	22.0	4	23.5	7	21.2	
T3	0	0.0	0	0.0	0	0.0	
T4	2	4.0	0	0.0	2	6.1	
Tis	2	4.0	1	5.9	1	3.0	
Total	50	100.0	17	34.0	33	66.0	

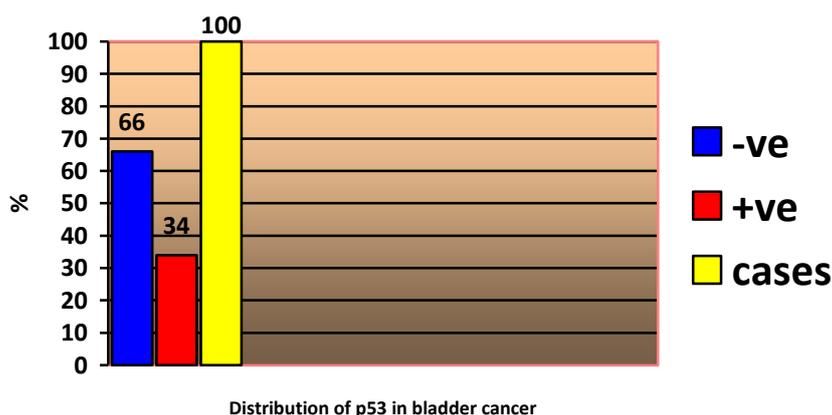
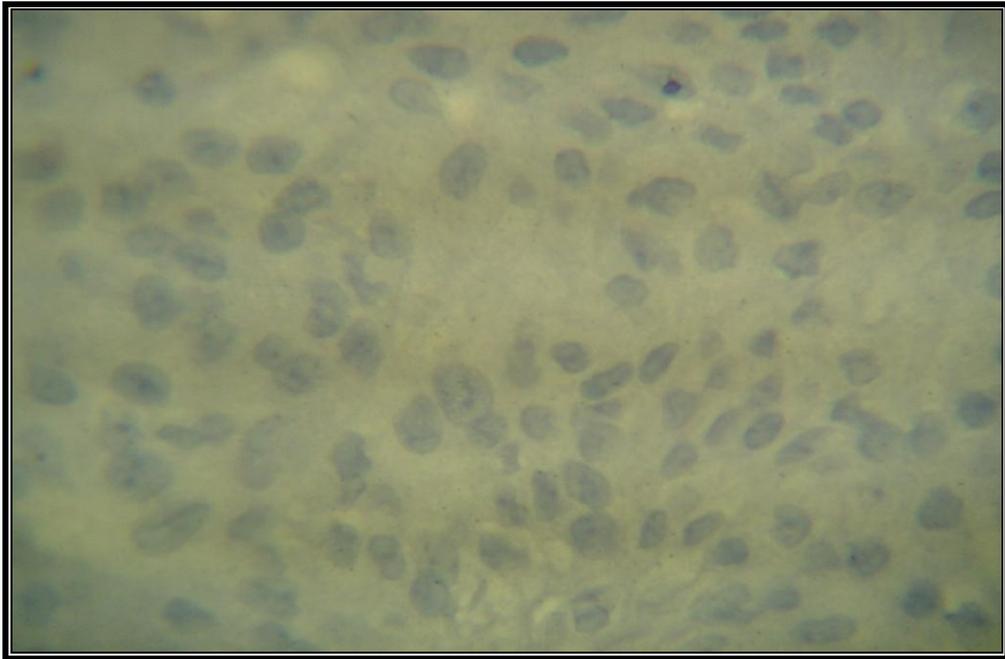


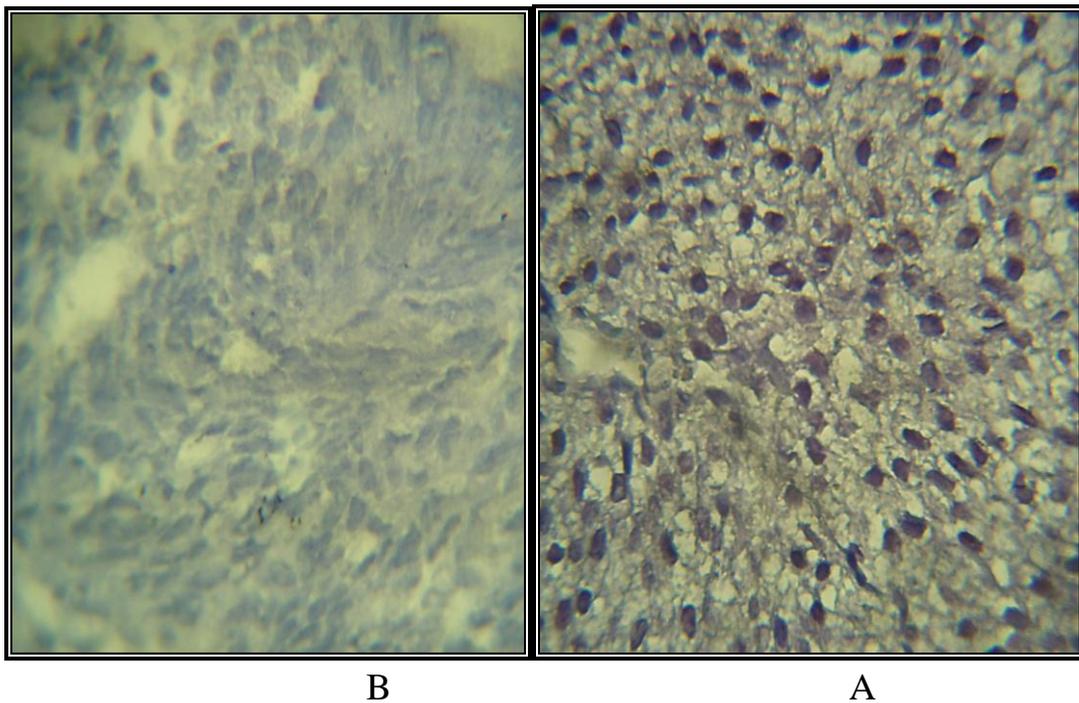
Figure (1): P53 status in urothelial tumors

Table (5): Frequency of P53 over-expression in urothelial carcinoma of the bladder in different studies.

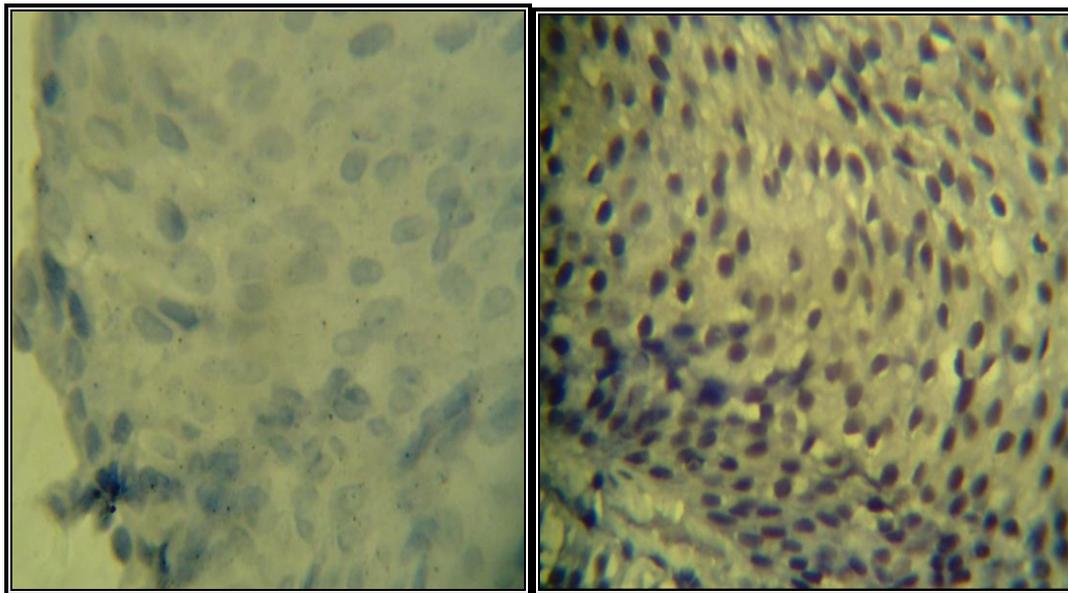
Study	Year	Site	NO. of Cases	% of P53 +Ve Cases
Current study	2012	Mosul	50	34%
Venyo A. et al. <sup>(21)</sup>	2010	U.K.	86	54%
Turk N. et al. <sup>(22)</sup>	2009	Turkey	84	47.6%
Ibrahim N. et al. <sup>(23)</sup>	2009	Egypt	74	74.3%
El-chennawi F. et al. <sup>(24)</sup>	2009	Egypt	50	66%
Abdul-Hameeda. et al. <sup>(19)</sup>	2007	Karbala-Iraq	58	50%
Galmozzi F. et al. <sup>(25)</sup>	2006	Italy	82	73.17%
Comperat E. et al. <sup>(26)</sup>	2005	France	158	63.9%
Serdar A. et al. <sup>(27)</sup>	2005	Turkey	61	64%
Cheng H. et al. <sup>(16)</sup>	2001	China	142	19%
Barsoum H. et al. <sup>(20)</sup>	2000	Egypt	49	67.3%
Shiina H. et al. <sup>(17)</sup>	1999	Japan	84	20.5%
Okamura T. et al. <sup>(7)</sup>	1998	Japan	79	42%
Sink Z. et al. <sup>(14)</sup>	1996	Turkey	44	18.2%



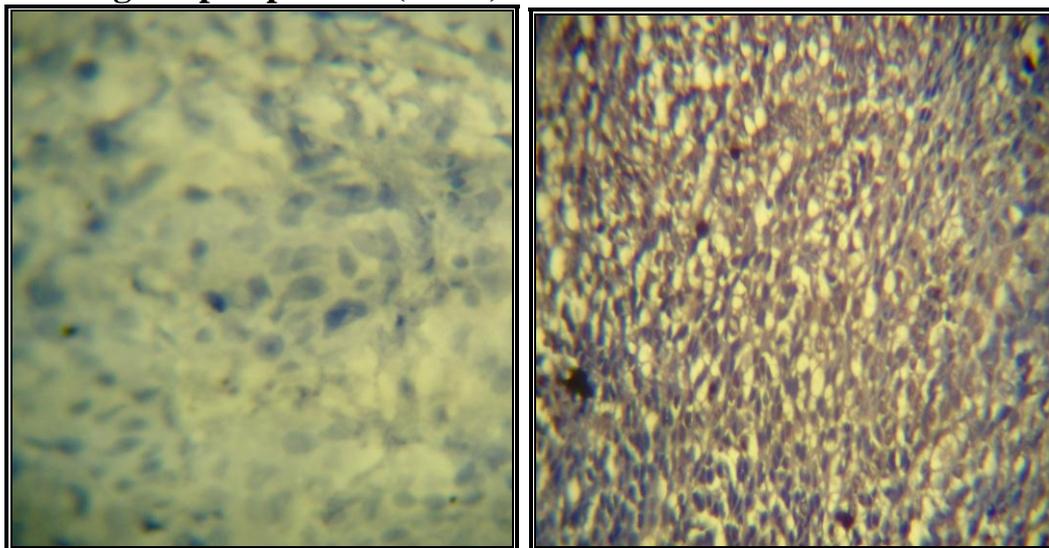
**Figure (2): IHC (Papilloma) negative staining for p53 protein (x 400).**



**Figure (3): IHC, PUNLMP, (A)negative staining, (B) positive staining for p53 protein (x 400).**



**Figure (4): IHC, Low grade, (A)negative staining, (B) positive staining for p53 protein (x 400).**



**Figure (5): IHC, High grade, (A)negative staining, (B) positive staining for p53 protein (x 400).**

## الخلاصة

### الأهداف:

في أورام المثانة في مدينة p53 تم إجراء هذه الدراسة لتقييم حالة الظهور المناعي لبروتين الموصل و الربط بينها وبين مختلف الصفات المرضية السريرية للورم و مقارنة هذه النتائج مع نتائج لدراسات أخرى.

### الحالات والطرق:

إن هذه الدراسة مستقبلية ورجعية تم من خلالها جمع ٥٠ حالة من أورام المثانة. تم جمع هذه الحالات من مستشفى الجمهوري التعليمي خلال فترة ٨ أشهر امتدت من شهر تموز ٢٠١١ إلى شهر شباط ٢٠١٢. وتم تصنيف الأورام وفقا لنظام تصنيف منظمة الصحة العالمية.

### النتائج والاستنتاجات:

لقد تراوحت أعمار المرضى بين ٢٣ و ٩١ سنة. وتم تقسيم الحالات السرطانية نسيجيا إلى حالة واحدة نوع ورم المثانة الحليمي و ١١ حالة لسرطان المثانة الحليمي للإمكانية الخبيثة المنخفضة و ٢٣ حالة لسرطان المثانة من نوع الدرجة المنخفضة و ١٥ حالة لسرطان المثانة من نوع الدرجة العالية.

في ١٧ حالة (٣٤%) من حالات سرطان المثانة. p53 من خلال الدراسة ظهر بروتين تراوحت بين p53 هناك دراسات أخرى مشابهة قد أظهرت نسب مختلفة لظهور بروتين (18.2% - 74.3%).

وعمر المريض او جنسه او درجة او p53 لم يكن هناك علاقة معنوية بين ظهور بروتين (بالتعاقب). P= 0.426، P=0.152، P=0.765، P=0.107 مرحلة الورم