Introduction

Bladder cancer (BLCA) is one of the most common malignancies that occur at any age and can affect both sexes. However, it affects predominantly middle-aged and elderly men. In males, it ranks as the fourth most common type of cancers after prostatic, lung and colorectal cancers, accounting for 6.6% of all cases of cancer [1]. In females, it is the ninth commonest cancer, constituting 2.4% of all cancers [1]. Bladder carcinoma contributes to 3.0% of all cancer-related mortality in men and 1.5% in women. The 5-year survival rates are higher in men than in women.

ABSTRACT

BACKGROUND: Carcinoma of urinary bladder is one of the most common malignancies worldwide and constitutes a major health problem. Multiple risk factors are associated with this tumor and its prognosis will depend on different clinicopathological parameters. Over expression of P53 protein and mutant Rb gene is associated with more aggressive clinical and histopathological features of the tumor such as advanced stage and higher grade.

AIM: The immunohistochemical expression of Rb gene and P53 gene will be assessed through their protein products in transitional cell carcinoma (TCC) of the urinary bladder and then will be correlated with other well-known risk factors and prognostic parameters of bladder TCC, such as grading, tumor size, smoking, alcohol drinking, and family history.

METHODS: Patients were recruited from the uro-surgical department/Surgical Subspecialties Teaching Hospital during the period from November 2020 to April 2021. In this study, patients enrolled were those suspected to have bladder carcinoma. The work up included a full history and clinical examination. Surgical samples were taken from the patients for histopathological evaluation; the study’s samples represented either endoscopic cup biopsy, transurethral resection of the tumor, or radical cystectomy. Sections obtained from these samples were stained with the conventional hematoxylin and eosin stain. Then, immunohistochemical staining for P53 and pRB was applied only for patients diagnosed with TCC.

RESULTS: The differences between low-grade and high-grade tumors regarding pRB percentage score were statistically significant (p = 0.026), but were not significant regarding the intensity score (p = 0.094). There were significant correlations between tumor stage and both pRB intensity and percentage scores (p = 0.044 and 0.042, respectively). Differences between low-grade and high-grade tumors regarding p53 intensity score were significant (p = 0.022). The differences between low-grade and high-grade tumors regarding p53 percentage score were significant (p = 0.049). The differences between different tumor stages regarding p53 intensity score were significant (p = 0.018). The differences between different tumor stages regarding P53 percentage score were significant (p = 0.019).

CONCLUSIONS: Tumor’s grade was found to be correlated with the tumor stage with no correlation with the age, gender, smoking, family history of TCC, history of urinary tract infection, bladder stones, nor the recurrence of the tumor. The pRB intensity and the percentage scores were correlated to each other and to tumor’s grade and stage, except for the pRB intensity which showed no correlation with the tumor’s grade. The P53 intensity and percentage scores were correlated to each other and also to tumor’s grade and stage, so that P53 is over-expressed in tumors with higher grade and stage.
deletions of these so-called cancer suppressor genes will encourage an unregulated growth and failure to direct cells with damaged DNA towards programmed cell death, resulting in uncontrolled proliferation of genetically unstable clones. Several tumor suppressor genes loci have been closely related to BLCA. These include retinoblastoma (Rb) gene on chromosome 13q14 and p53 on chromosome 17p13 [2], [4].

Progression through cell-cycle is regulated by complex molecular pathway involving cyclins, cyclin-dependent kinases (CDK), and CDK inhibitors (CDKIs) [5]. The Rb and p53 growth suppression genes play an essential role in the control of the cell cycle [5]. P53-dependent G1-S and G2-M cell-cycle arrest is mediated in part, through p53-mediated activation of the CDKI p21 and suppression of the promoters of cyclins B1 and CDK1, respectively [6]. The Rb pathway is involved in the inhibition of transcription of genes necessary for the G1-S transition [6]. Hypophosphorylated Rb protein binds to and inactivates the transcription factors, notably the E2F1, that is important for the G1-S transition. As the cycle progresses, pRb becomes hyperphosphorylated and loses its ability to bind to and inactivate E2F1. The phosphorylation of pRb is done by the cyclin D-CDK4/6 complexes and is inhibited by the CDKI p16 [5].

Abnormalities in the cell-cycle components are common in urinary bladder urothelial carcinomas (BUC) and may be related to the pathogenesis and clinical behavior of these tumors [5]. P53 mutation and/or protein overexpression was significantly related to tumor grade, stage and the presence or absence of lymph node metastases [5].

The Rb protein is a major tumor suppressor, controlling cellular processes and responses to oncogenic stimuli, like DNA damage, repeated rapid cells division, as well as inappropriate mitogenic signal [7], [8]. The importance of Rb protein in tumor development was first shown by the results of RB allele being always deleted in Rb [9]. Altered pRb protein expression is present in all grades and stages of bladder urothelial carcinoma and is more commonly seen in higher grade and muscle invasive tumors [5]. The immunohistochemical overexpression of pRb protein has been linked with increased rate of tumor progression and decreased survival [5]. aberrant expression of p16 and cyclin D1 proteins had been related to urothelial carcinogenesis and tumor recurrence [10], [11], [12].

About ninety % of urinary bladder malignancies are urothelial cell carcinomas, which are broadly grouped into muscle-invasive bladder carcinoma (MIBC) and non-MIBC (NMIBC) [13]. MIBC usually presents with a poor prognosis, and this represents more than 50% of mortality account for their disease [14]. On the other hand, patients with NMIBC generally have a variable clinical behavior with potential for progression and significant risk of recurrence [15]. The International Society of Urological Pathology (ISUP) meeting in 2013 declared that there is no ideal marker with respect of urothelial differentiation [16]. However, in recent years, there have been a great effort in biomarkers in the prognosis and prediction of BLCA, such as protein 53 (p53), protein 21 (p21), RB transcriptional corepressor (pRB), and so on [17]. Even more useful is the probability of finding a precise biomarker that could be applied to the routine clinical practice to evaluate the clinical outcome by immunohistochemistry [18].

Aims of the study
The aim of the study is as follows:

1. To study immunohistochemical expression of Rb gene through its protein product (pRb) and P53 gene through its protein product in transitional cell carcinoma (TCC) of urinary bladder.
2. To correlate between such expressions and other well-known risk factors and prognostic parameters of the bladder TCC, such as grade, stage, smoking, alcohol drinking, history of urinary tract infection (UTI) or lithiasis, and family history.

Patients, Materials and Methods

Selection of patients
In this prospective study, the patients were recruited from the urosurgical department/Surgical Subspecialties Teaching Hospital from November 2020 through April 2021.

Sixty-four patients suspected to have bladder carcinoma were examined endoscopically under general anesthesia by their urologists. Of these, 51 patients had bladder mass, which was either resected (alone or as a part of radical cystectomy) or biopsied, whereas thirteen had no detectable mass. The latter group was not followed further. Of the remaining 51 patients with bladder mass lesion, the biopsy results disclosed in further thirteen patients, cystitis (and its associated mucosal nodularity and edema) without evidence of tumor. This left us with thirty eight, whose biopsy histology confirmed the presence of carcinomas. The carcinomas were transosinal in 34 patients, adenocarcinoma in two patients, squamous cell carcinoma in one patient, and small cell carcinoma in one patient. Those with non-TCC (four patients) were excluded from further follow-up: the remaining 34 patients were supposed to constitute the core of our study. Regrettably, the histological blocks of two patients were taken by their relatives to get a second consultation opinion regarding the diagnosis; these blocks were never returned back at least during the period of the study. Thus, our study was concentrated
on 31 patients only. Clinical information regarding name, age, gender, residence, symptomatology, as well as specific questions regarding family history of similar cancer, smoking and drinking habits, history of UTI and renal stones, etc., was obtained either directly from the patient or from the hospital records. The results of physical examination, imaging studies and other relevant particulars were obtained from the case notes of the patients or through direct communication with the respective specialist in charge of the case, and we analyzed our work as follow:

**Study group**

In this study, 64 patients were recruited; they were suspected of having urinary bladder carcinoma. The work up included full history and clinical examination. Surgical samples were taken from 51 patients for histopathological evaluations; the samples were either endoscopic cup biopsy, transurethral resection of the tumor, or radical cystectomy. Sections obtained from the forementioned samples were stained with conventional hematoxyline-eosine stain. Immunohistochemical staining for P53 and pRB was then done for the 31 patient samples diagnosed with TCC.

**Positive control**

These comprise samples of adenocarcinoma of the lung; positive staining of the tumor cells was considered as a positive control for P53.

Similarly, specimens of small cell carcinoma of the lung were treated with primary antibody of pRB; positive staining of the small cell carcinoma cells was considered as a positive control for pRB.

**Negative control**

Additional sections from the study group were treated identical to the sections under investigation but with the omission of the primary antibody (pRB) or (P53) and were considered as negative controls for each set of slides.

**Histological and immunohistochemical workup**

**Sample preparation**

Paraffin-embedded tissue blocks of the collected samples as well as the control groups were prepared. Sections were made from each of the paraffin embedded blocks and as follows:

- Eight, 4 μm-thick sections were cut from each paraffin block
- Four sections were subjected for hematoxylin and eosin staining. Ordinary non-charged glass slides were used. This was conducted to evaluate the cases microscopically.
- The other four sections were used for immunohistochemical staining procedures to detect P53 and pRB. In this instance positively charged slides were used.

**pRB tumor marker**

**Specific reagents**

- Immunohistochemistry detection kit, Mouse IgG, Bioassay (United States Biological, USA), and antibody (pRB tag antibody). The kit contains the followings:
  - Anti-Mouse IgG (Biotin).
  - DAB, DAB Buffer and DAB Detoxification Reagent
  - Normal serum
  - Solution A and B
  - Rb antibody: MBI IH-60030, Human pRb Biotin DNA L.N. 22057095.

**General reagents and sources**

All are listed in Table 1.

### Table 1: General reagents and solutions used throughout the study

| General reagents and solutions | Brand |
|-------------------------------|-------|
| Absolute alcohol | BDH (England) |
| Counter stain (hematoxylin and Nuclear Fast Red) | Hopkins and Williams |
| Distilled water | |
| Mounting medium | DPX (BDH, England) |
| Xylene | Merck (Germany) |
| Phosphate buffered saline (PBS) | OXIOD (England) |

Principle of the test

A biotinylated, cross-adsorbed, and affinity purified secondary anti-mouse IgG is used to detect primary antibody-antigen complexes adhered to a glass microscope slide, following reaction with an enhanced detection reagent.

### Preparation of tissue sections and reagents

1. Paraffin-embedded tissue sections were cut 4 μm thick, floated in protein-free water bath, and then placed on Biocare techniques starfrost slides, left at room temperature to dry and then in 55°C overnight.
2. Counter stain hematoxylin was prepared by dissolving 10 μg hematoxylin in 1000 ml distilled water to which 0.5 ml acetic acid and ½ thymol tablets were added.
3. Citric buffer was prepared by diluting 10 ml of 100 × concentrated citric buffer into distilled water to a final volume of 1000 ml.

4. Biotin was prepared by mixing 1.4 ml of 1 × Phosphate buffered saline (PBS), pH 7.4 and 35 µl of biotin in a tube.

5. Detection solution was prepared by mixing 35 µl of solution A and 35 µl of solution B in a tube and incubated at room temperature for 30 min before use.

6. Fresh development solution was made by mixing 1.6 ml of DAB Buffer and 35 µl of DAB in a tube.

7. Normal serum 1% was prepared by mixing 3.5 ml 1 × PBS with 35 µl of normal serum.

Immunohistochemistry procedure

The slides were incubated over night at 65°C in vertical position for deparaffinization then:

Day 1

- The slides were soaked in xylene twice, each time for 15 min.
- Dehydration by ethanol alcohol in the following order: 100% (I), 100% (II), 95%, 90%, 80%, and 70% for 5 min in each solution then in distilled water for 5 min.
- The slides were immersed in 0.3% H$_2$O$_2$ (in distilled water) for 30 min at room temperature.
- The slides were rinsed by distilled water followed by 1 × PBS (pH 7.4).
- Antigen retrieval was done by immersing the slides in citric buffer jar followed by placing the latter in a microwave oven set at 720 watt for 10 min.

Note: Four methods were tried for antigen retrieval which are:

A. Microwave oven for 10 min.
B. Autoclave for 2 min.
C. Water bath for 10 min set at boiling temperature.
D. No retrieval.

The best expression was obtained with the use of microwave oven.

- The tissue sections were circled by a Pap Pen.
- Incubation of the slides with 1% normal serum was done for 30 min at room temperature (25°C).
- Normal serum dropped off and overnight incubation with pRb antibody diluted (1:10) was performed.

Day 2

- The slides were rinsed 3 times with 1 × PBS for 5 min each.

Evaluation of Immunohistochemical protein expression

A biotinylated, cross-adsorbed, and affinity purified secondary anti-mouse IgG was used to detect primary antibody-antigen complexes adhered to a positively charged glass microscope slide, following reaction with an enhanced detection reagent, proper, and accurate application of kit instructions led to appearance of a brown nuclear precipitate in positive cells on tissue sections.

Readings were done double blindly by two pathologists; IHC was given intensity and percentage scores, based on intensity of positive staining and number of cells staining, respectively. A scale of 0–3 was used to measure relative intensity with 0 corresponding to no detectable IHC reaction and 1 equivalent to low, 2 equivalents to moderate, and 3 equivalents to high. Positive cells were counted in ten different fields for each samples and the average of positive cells of the ten fields was determined assigning cases to one of the 4 following categories:

i. Score 1: 1–25%.
ii. Score 2: 26–50%.
iii. Score 3: 51–75%.
iv. Score 4: 76–100% [19].

For the intensity of staining, an intensity score of >1 was assigned as high and a percentage score of >3 was categorized as high [19].

P53 tumor marker

Solutions and Reagents

Primary antibodies: Monoclonal Mouse Anti-Human P53 Protein, 11 ml, Ready-To-Use,
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DAKO, Clone DO-7, Code M7001, LOT 00005848, Dakopatts Corporation, USA.

b. Antibody Diluent with Background Reducing Components, 125ml, Code S0809, LOT 00002288, Dako North America, with a dilution of 1:25.

c. Antigen retrieval: Target Retrieval Solution, 500 ml, Code S2368, LOT 00026677, Dako Denmark A/S Produktionsvej 42 DK-2600 Glostrup, phosphate buffer solution, pH 6.0 for P53.

d. Buffer solution: Phosphate buffer for P53.

e. Staining kit: DakoCytomation, Code K0673, sufficient for 150 tissue sections, which includes: Peroxidase Block, 1 × 15 ml, 3% hydrogen peroxide in water.

1. Biotinylated Link, 1 × 15 ml, biotin labeled affinity isolated goat anti-rabbit and goat anti-mouse immunoglobulins in PBS, containing stabilizing protein and 0.015 mol/L sodium azide.

2. Streptavidin-HRP: 1 × 15 ml, Streptavidin conjugated to horseradish peroxidase in PBS containing stabilizing protein and anti-microbial agents.

3. DAB substrate buffer, 1 × 18 ml, Imidazole-HCL buffer PH 7.5 containing hydrogen peroxide and an anti-microbial agents.

4. DAB chromogen, 1 × 1 ml, 3,3’-diaminobenzidine in chromogen solution.

5. Accessories: Calibrated test tube, plastic Pasteur pipette.

Immunohistochemical staining protocol

Technique

The Avidin Biotin Complex detection system was used on specimens of five microns thick sections that were cut from the formalin-fixed, paraffin-embedded blocks and placed on positively charged slides.

- Deparaffinization by heating the slides in an oven at 65°C for 60 min followed by two changes of xylene for 10 min each.
- Rehydration done through serial alcohol concentrations of 100%, 95%, 90%, 80%, and 70%, each for 5 min and then in distilled water for another 5 min.
- Immersion in retrieval (phosphate buffer, pH 6.0 for P53), by heating in the microwave at low heat for 20 min then medium heat for 15 min then high heat for 10 min.
- Sections were let to cool at room temperature (for 30 min).
- Bathing in PBS (for 2–5 min).
- Slides were tapped of excess buffer followed by wiping around the sections by gauze pad. A circle was made around the sections by a PAP pen.

- Enough hydrogen peroxide was applied (10 min).
- Sections were washed in a phosphate buffer, tapped of excess buffer; the slide was wiped around the sections.
- Enough primary antibodies were applied (for 60 min).
- The sections were washed and excess fluid was removed by wiping around the sections.
- A biotinylated link was applied (30 min).
- Sections were washed and wiped around.
- Streptavidin-peroxide was applied for (30 min).
- Sections were then wash and wiped around.
- Substrate-chromogen was then applied for (5–15 min).
- Sections where then washed in distilled water
- Sections were counter stained with Meyer’s hematoxylin then rinsed in tap water, mounted with mounting medium, and covered with cover slips examined under light microscope.

Scoring system

The criterion for positive immunoreactions for P53 is a dark brown nuclear precipitate. Quantification of p53 protein expression was evaluated under light microscopy at low power ×40 and ×100 (×4, and ×10 objectives respectively an × 10 eye pieces), whereas the counting of positive cells (nuclear staining) was performed at oil emersion (×100).

Each sample was scanned for at least ten fields with a high power magnification.

The scoring was done according to [20], at 40 × objective as follow:

Score (0): Negative, none of the cells are positive for the marker.
Score (+1): Mild or weak staining; 5–10% of the cells are positive for the marker.
Score (+2): Moderate staining; <25% of the cells are positive for the marker.
Score (+3): Strong staining; 25–50% of the cells are positive for the marker.
Score (+4): Highly strong staining, over 50% of tumor cells are stained positive.

Qualitative assessment

Faint staining pattern, whether cytoplasmic or nuclear, that could only be detected by using higher magnification (objective 40). Strong staining pattern is easily seen by low magnification (objective 4).

Photography

Pictures were taken by adjusting a digital camera lens on the eye piece of the microscope (oil immersion, ×1000, and ×400). Sony Cyber Shot
digital camera with 8.1 mega pixels was used and the files were saved in joint photographic experts group format.

Statistical analysis
Statistical analysis was performed with SPSS 16 for windows evaluation version and the Microsoft Office Excel (2007). Univariate data were summarized using standard descriptive statistics, tabulation of the categorical variables and histograms of the numerical variables. Correlations between the categorical variables were assessed through cross tabulations and Chi-squares. In all statistical analyses, p < 0.05 was considered to be significant.

Results

Statistical results
Clinicopathological data
Age distribution
The age range of the studied patients was 28–85 years old with the mean age 60.2 years and median age of 67 years. The majority of the studied patients were in the age group of 61–70 years (13, (40.6%), whereas the minority was in the age groups of 21–30 years and 31–40 years (1), (3.1%). The rest of the patients were distributed in the age groups below as shown in Table 2.

Table 2: Age distribution of the 32 patients with TCC

| Age groups (years) | Frequency | Percent | Cumulative Percent |
|-------------------|-----------|---------|--------------------|
| 21–30             | 1         | 3.1     | 3.1                |
| 31–40             | 1         | 3.1     | 6.2                |
| 41–50             | 4         | 12.5    | 18.8               |
| 51–60             | 6         | 18.8    | 37.5               |
| 61–70             | 13        | 40.6    | 78.1               |
| 71–80             | 5         | 15.6    | 93.8               |
| 81–90             | 2         | 6.2     | 100.0              |
| Total             | 32        |         |                    |

Gender distribution
In this study, 22 patients (68.8%) were males and ten patients (31.2%) were females as shown in Table 3.

Table 3: Gender distribution in 32 patients with transitional cell carcinoma of the bladder

| Gender | Frequency | Percent | Cumulative Percent |
|--------|-----------|---------|--------------------|
| Male   | 22        | 68.8    | 68.8               |
| Female | 10        | 31.2    | 100.0              |
| Total  | 32        |         |                    |

Smoking
Of the total 32 patients, 19 (59%) were smokers, the rest were nonsmokers as shown in Table 4.

Table 4: Smoking habit in 32 patients with transitional cell carcinoma of the bladder

| Smoking history | Frequency | Percent | Cumulative Percent |
|-----------------|-----------|---------|--------------------|
| Non smokers     | 13        | 40.6    | 40.6               |
| Smokers         | 19        | 59.4    | 100.0              |
| Total           | 32        |         |                    |

Amount of smoking
The number of the cigarettes smoked of those 19 smokers differed widely; they ranged from 1 to 5 cigarettes/day up to 61 to 80 cigarettes/day. The full data regarding smoking habit are tabulated in Table 5.

Table 5: Amount of cigarettes smoking in 32 patients with transitional cell carcinoma of the bladder

| Amount of smoking | Frequency | Percent | Cumulative Percent |
|-------------------|-----------|---------|--------------------|
| Not smoker        | 13        | 40.6    | 40.6               |
| 1–5 cigarettes    | 1         | 3.1     | 43.8               |
| 6–10 cigarettes   | 3         | 9.4     | 53.1               |
| 11–20 cigarettes  | 7         | 21.9    | 75.0               |
| 21–30 cigarettes  | 1         | 3.1     | 78.1               |
| 31–40 cigarettes  | 4         | 12.5    | 90.6               |
| 41–60 cigarettes  | 2         | 6.2     | 96.9               |
| 61–80 cigarettes  | 1         | 3.1     | 100.0              |
| Total             | 32        |         |                    |

Duration of smoking
The duration of smoking in those 19 smoking patients also varied widely and ranged from (6 years to 58 years). Full information regarding duration of smoking is shown in Table 6.

Table 6: Duration of smoking of the 19 smokers with transitional cell carcinoma of the bladder

| Duration of smoking (years) | Frequency | Percent | Cumulative Percent |
|-----------------------------|-----------|---------|--------------------|
| 0–10                        | 1         | 5.26    | 5.26               |
| 11–20                       | 5         | 26.31   | 31.57              |
| 21–30                       | 4         | 21.05   | 52.62              |
| 31–40                       | 7         | 36.84   | 89.46              |
| 41–50                       | 1         | 5.26    | 94.72              |
| 51–60                       | 1         | 5.26    | 96.9               |
| Total                       | 19        |         | 100.0              |

Association with UTI
History of (UTI) in those 32 patients with TCC revealed that 18 (56.2%) patients have had no history of (UTI), while 14 (43.8%) patients have had such a history as shown in Table 7.

Table 7: Frequency distribution of the 32 patients with and without a history of UTI

| History of UTI | Frequency | Percent | Cumulative Percent |
|----------------|-----------|---------|--------------------|
| No UTI         | 18        | 56.2    | 56.2               |
| UTI            | 14        | 43.8    | 100.0              |
| Total          | 32        |         |                    |

Family history of urethelial carcinoma
Of the 32 patients with TCC of the urinary bladder only three patients (9.4%) had a positive family history of TCC; this is shown in Table 8.

Table 8: Frequency of patients with a family history of TCC of urinary bladder

| Family history of bladder cancer | Frequency | Percent | Cumulative Percent |
|---------------------------------|-----------|---------|--------------------|
| Negative for family history     | 29        | 90.6    | 90.6               |
| Positive for family history     | 3         | 9.4     | 100.0              |
| Total                           | 32        |         |                    |
Association with urinary lithiasis

Of the 32 patients with TCC, five patients (15.6%) have had bladder stones; this is shown in Table 9.

Table 9: Relative frequency of those with TCC of the bladder with a history of bladder stone

| History of stone | Frequency | Percent | Cumulative Percent |
|------------------|-----------|---------|--------------------|
| History of stone | 27        | 84.4    | 84.4               |
| No history of stone | 5      | 15.6    | 100.0              |
| Total            | 32        | 100.0   |                     |

History of alcohol intake

Only four patients out of the total (12.5%) confessed of drinking habits. This is shown in Table 10.

Table 10: Frequency of patients with a positive history of alcohol intake among the 32 patients with TCC

| History of alcohol drinking | Frequency | Percent | Cumulative Percent |
|-----------------------------|-----------|---------|--------------------|
| Not drinker                 | 28        | 87.5    | 87.5               |
| Drinker                     | 4         | 12.5    | 100.0              |
| Total                       | 32        | 100.0   |                     |

Tumor recurrence

Of the total 32 patients with TCC of the urinary bladder 17 patients have had a previous documented history of similar tumors, that is, during this study they were in reality cases with a recurrent disease. This is shown in Table 11.

Table 11: Relative frequency of recurrent TCC

| History of TCC | No. of patients | Percent | Cumulative Percent |
|----------------|-----------------|---------|--------------------|
| Primary cases  | 15              | 46.9    | 46.9               |
| Recurrent cases| 17              | 53.1    | 100.0              |
| Total          | 32              | 100.0   |                     |

Tumor grade

Histopathological assessment of the tumor grade in the 32 patients with TCC revealed that (14) patients had low-grade tumors whereas the remaining (18) had high-grade ones (Figures 1 and 2). This is shown in Table 12.

Table 12: Relative frequency of TCC cases with respect to the grade

| Tumor grade | Frequency | Percent | Cumulative Percent |
|-------------|-----------|---------|--------------------|
| Low grade   | 14        | 43.8    | 43.8               |
| High grade  | 18        | 56.2    | 100.0              |
| Total       | 32        | 100.0   |                     |

Tumor stage

Staging of the studied tumors according to TNM staging system revealed that the majority of the cases had T1a tumors (18 patients; 56.2%) followed by T1b tumors (6 patients; 18.8%). This is shown in detail in Table 13. It should be mentioned that only one patient (T4a) of the total showed lymph node enlargement through imaging techniques (N1), and that none of the cases had distant metastases documented by the imaging studies (M0).

Table 13: Relative frequency of TCC according the TNM staging

| Tumor stage | Frequency | Percent | Cumulative Percent |
|-------------|-----------|---------|--------------------|
| Tis          | 1         | 3.1     | 3.1                |
| T1a          | 18        | 56.2    | 59.4               |
| T1b          | 6         | 18.8    | 78.1               |
| T2a          | 1         | 3.1     | 81.2               |
| T3a          | 2         | 6.2     | 87.5               |
| T3b          | 2         | 6.2     | 93.8               |
| T4aN0Mx      | 1         | 3.1     | 96.9               |
| T4aN1Mx      | 1         | 3.1     | 100.0              |
| Total        | 32        | 100.0   |                     |

Correlations between different clinicopathological data

In this study, a correlation is attempted between tumor grade and some clinicopathological data such as age, gender, smoking habit, history of UTI, history of renal stone, family history of TCC, recurrence of the tumor, and tumor stage.

Correlation between tumor grade and age

In this study, high-grade tumors were more commonly encountered than low-grade tumors (18 patients vs. 14). The highest incidence of low-grade tumors was in the age group 61–70 years (6 patients of the 32); and the highest incidence of high-grade tumors was also in this very age group (7 patients out of the 32). The frequency of low- and high-grade urothelial carcinomas with respect to the various age groups is detailed in Table 14. Hence, the correlation between tumor grade and patients age was not significant since p = 0.101.

Table 14: Correlation between age groups and tumor grade

| Age (years) | Low grade | High grade | Total |
|------------|-----------|------------|-------|
| 21–30      | 1         | 12         | 13    |
| 31–40      | 0         | 1          | 1     |
| 41–50      | 1         | 3          | 4     |
| 51–60      | 3         | 3          | 6     |
| 61–70      | 6         | 7          | 13    |
| 71–80      | 3         | 2          | 5     |
| 81–90      | 0         | 2          | 2     |
| Total      | 14        | 18         | 32    |

p = 0.701 (Not significant).

Correlation between tumor grade and gender

This study comprises 22 males and ten females. Ten out of the 22 males had low grade TCC (45.5%). On the other hand, only four out of the ten females had low grade TCC (40%). This is detailed in Table 15. Such correlations, however, were not statically significant. (p = 0.288).

Table 15: Correlation between tumor grade and gender

| Gender | Low grade | High grade | Total |
|--------|-----------|------------|-------|
| Male   | 10        | 12         | 22    |
| Female | 4         | 6          | 10    |
| Total  | 14        | 18         | 32    |

p = 0.288 (Not significant).
Correlation between tumor grade and smoking

Of the 32 urothelial carcinomas studied, smokers outnumbered nonsmokers (59.3% vs. 40.6%). In addition, smokers displayed slightly more relative frequency of high-grade tumors than nonsmokers (34.3% vs. 21.8%) out of the 32 patients. This is detailed in Table 16. However, statistically the above associations were not significant.

Table 16: Correlation between smoking habit and tumor grade

| Smoking-Tumor grade Cross tabulation | Tumor grade | | | |
| --- | --- | --- | --- | --- |
| | Low grade | High grade | Total | |
| Non smoker | 6 | 7 | 13 | |
| Smoker | 8 | 11 | 19 | |
| Total | 14 | 18 | 32 | |

*p = 0.275 (Not significant).

Correlation between tumor grade and UTI

In this study, the number of patients with urothelial carcinoma but no history of UTI outnumbered those with UTI (18 vs. 14). However, the relative frequency of high-grade tumors is higher in those with a history of UTI than those without such history, (31.2% vs. 25%). These associations are detailed in Table 17. There was no significant difference since the *p* = 0.093.

Table 17: Correlation between tumor grade and history of UTI

| History of UTI-Tumor grade Cross tabulation | Tumor grade | | | |
| --- | --- | --- | --- | --- |
| | Low grade | High grade | Total | |
| No UTI | 10 | 8 | 18 | |
| Has UTI | 4 | 10 | 14 | |
| Total | 14 | 18 | 32 | |

*p = 0.093 (Not significant).

Correlation between tumor grade and family history

Eleven patients out of 29 who had no family history for TCC with low grade and 18 with high grade TCC, while three patients who had positive family history all with low grade TCC as shown in Table 18. There was no significant correlation between the two variables since *p* = 0.073.

Table 18: Correlation between family history and tumor grade

| Family History-Tumor grade Cross tabulation | Tumor grade | | | |
| --- | --- | --- | --- | --- |
| | Low grade | High grade | Total | |
| Negative for family history | 11 | 19 | 29 | |
| Positive for family history | 3 | 0 | 3 | |
| Total | 14 | 18 | 32 | |

*p = 0.073 (Not significant).

Correlation between tumor grade and history of bladder stone

Those with a history of bladder stones displayed a higher relative frequency of high-grade tumors than those without (9.3% vs. 6.25%) out of the 32 patients. The various associations are detailed in Table 19. There was no significant association between tumor grade and bladder stone since *p* = 0.369.

Table 19: Correlation between history of bladder stones and tumor grade.

| History of bladder stone-Tumor grade Cross tabulation | Tumor grade | | | |
| --- | --- | --- | --- | --- |
| | Low grade | High grade | Total | |
| No stone | 12 | 15 | 27 | |
| Has a stone | 2 | 3 | 5 | |
| Total | 14 | 18 | 32 | |

*p = 0.36 (Not significant).

Correlation between tumor grade and recurrence of tumor

Those patients with recurrent tumors displayed slightly higher relative frequency of high-grade carcinomas than those patients with primary tumors (55.5% vs. 44.5%) out of 18 patients with high grade tumor. The correlation is not, however, statistically significant (*p* = 0.265). The relationship between these two parameters is detailed in Table 20.

Table 20: Correlation between tumor grade and recurrence of malignancy

| Primary or recurrent cases-Tumor grade Cross tabulation | Tumor grade | | | |
| --- | --- | --- | --- | --- |
| | Low grade | High grade | Total | |
| Primary cases | 7 | 8 (44.5%) | 15 | |
| Recurrent cases | 7 | 10 (55.5%) | 17 | |
| Total | 14 | 18 (100%) | 32 | |

*p = 0.265 (not significant).

Correlation between tumor grade and stage

All the (14) patients with low-grade tumors, in this study, show superficial invasion, that is, limited to mucosa/submucosa (T1a or T1b); however, seven of the eighteen patients (38.9%) with high-grade tumors presented in advanced stages, that is, T2 or more. This finding was found to be statistically significant with *p* = 0.003. The relationship between tumor grade and stage is detailed in Table 21.

Table 21: Correlation between tumor grade and stage

| Tumor grade-Tumor stage Cross tabulation | Tumor stage | | | |
| --- | --- | --- | --- | --- |
| | T1S | T1a | T1b | T2a | T3a | T3b | T4aN0Mx | T4aN1Mx | Total | |
| Low grade | 1 | 10 | 3 | 0 | 0 | 0 | 0 | 14 | |
| High grade | 0 | 8 | 3 | 1 | 2 | 2 | 1 | 1 | 18 | |
| Total | 1 | 18 | 6 | 1 | 2 | 2 | 1 | 1 | 32 | |

*p = 0.003 (significant).

Immunohistochemical scores

PRb scores

Two patients out of the total 32 showed negative PRb intensity and percentage scores. The remaining 30 patients showed variable intensity and percentage scores (Figure 3).

Intensity scores

Half of the patients studied displayed low intensity scores, whereas about one-third revealed moderate intensity. Rare carcinomas showed high intensity. These findings are detailed in Table 22.
Table 22: Frequency distribution of various PRb intensity scores

| PRb intensity score | Frequency | Percent | Cumulative Percent |
|---------------------|-----------|---------|--------------------|
| Negative staining   | 2         | 18.8    | 18.8               |
| Low intensity       | 16        | 50.0    | 68.8               |
| Moderate intensity  | 11        | 34.4    | 103.2              |
| High intensity      | 3         | 9.4     | 100.0              |
| Total               | 32        | 100.0   |                     |

Percentage scores

The results of the percentage scores parallel those of the intensity in that almost half of the patients revealed +1 score, about one-third revealed +2 score. +3 score was rarely reported. These results are detailed in Table 23.

Table 23: Frequency distribution of various PRb percentage scores of the 32 urothelial carcinomas

| PRb percentage score | Frequency | Percent | Cumulative Percent |
|----------------------|-----------|---------|--------------------|
| Negative 0%          | 2         | 6.2     | 6.2                |
| 1–25% (+1)           | 15        | 46.9    | 53.1               |
| 26–50% (+2)          | 11        | 34.4    | 87.5               |
| 51–75% (+3)          | 4         | 12.5    | 100.0              |
| Total                | 32        | 100.0   |                     |

P53 scores

Positive expression of p53 protein by immunohistochemistry was detected as brownish precipitate in the nucleus of the tumor cells and that was used in scoring of p53 expression.

Six patients (18.8%) revealed negative staining so negative for both intensity and percentage scores (Figure 4).

Intensity score

The intensity of expression of P53 varied from weak or mild up to very strong, with one-third of the tumors (34.4%) showing moderate intensity. This is detailed in Table 24.

Table 24: Distribution of P53 intensity score

| P53 intensity score | Frequency | Percent | Valid percent | Cumulative percent |
|---------------------|-----------|---------|---------------|--------------------|
| Negative intensity  | 6         | 18.8    | 18.8          | 18.8               |
| Weak or mild intensity | 16   | 18.8    | 37.5          | 56.2               |
| Moderate intensity  | 11        | 34.4    | 71.9          | 103.2              |
| Strong intensity    | 6         | 18.8    | 90.6          |                     |
| Highly strong intensity | 3  | 9.4     | 100.0         |                     |
| Total               | 32        | 100.0   |               |                     |

Percentage score

The percentage scores of the 32 cases studied ranged from negative for staining up to +3. About one-third of the cases (31.2%) showed a +1 score followed by one-fifth of the cases (21.9%) showing +2 score. This is detailed in Table 25.

Table 25: Frequency distribution of p53 percentage score

| P53 percentage score | Frequency | Percent | Cumulative Percent |
|----------------------|-----------|---------|--------------------|
| Negative for staining | 6         | 18.8    | 18.8               |
| 5–10% of tumor cells positive for staining (+1) | 10 | 31.2    | 50.0               |
| <25% of tumor cells positive for staining (+2) | 7  | 21.9    | 71.9               |
| 25–50% of tumor cells positive for staining (+3) | 6 | 18.8    | 90.6               |
| Over 50% of tumor cells positive for staining (+4) | 3 | 9.4     | 100.0              |
| Total                | 32        | 100.0   |                     |

Correlations between PRb scores and tumor grade

PRb intensity score and tumor grade

The difference between low grade and high grade tumors regarding PRb intensity score was found to be statistically significant (p = 0.026) as shown in Table 27.

Table 27: Correlation between PRb percentage score and tumor grade

| PRb percentage score-Tumor grade Cross tabulation | Tumor grade |
|--------------------------------------------------|-------------|
| Low grade                                        | High grade  | Total |
| Negative staining                                | 2           | 0     | 2     |
| Low intensity                                    | 7           | 9     | 16    |
| Moderate intensity                               | 4           | 7     | 11    |
| High intensity                                   | 1           | 2     | 3     |
| Total                                            | 14          | 18    | 32    |

p = 0.026 (significant).

Correlations between p53 scores and tumor grade

Between p53 intensity score and tumor grade

The difference between low grade and high grade regarding p53 intensity score was significant (p = 0.022) as shown in Table 28.

Table 28: Correlation between p53 intensity score and tumor grade

| P53 intensity score-Tumor grade Cross tabulation | Tumor grade |
|--------------------------------------------------|-------------|
| Low grade                                        | High grade  | Total |
| Negative staining                                | 4           | 2     | 6     |
| Weak or mild staining                            | 3           | 3     | 6     |
| Moderate staining                                | 5           | 6     | 11    |
| Strong staining                                  | 2           | 4     | 6     |
| Highly strong staining                           | 0           | 3     | 3     |
| Total                                            | 14          | 18    | 32    |

p = 0.022 (significant).

Correlations between p53 percentage score and tumor grade

Between p53 percentage score and tumor grade

The difference between low grade and high grade urothelial carcinomas regarding p53 percentage scores was statistically significant (p = 0.049) as shown in Table 29.
Low grade
High grade

Correlation between pRb scores and tumor stage

Between pRb intensity score and tumor stage

The correlation between the percentage and intensity scores of pRb staining shows significant statistical difference since \( p < 0.0001 \) as shown in Table 34.

Correlation between p53 and percentage scores

The correlation between the percentage and intensity of p53 scores shows significant statistical differences \( p < 0.0001 \) as shown in Table 35.

### Table 29: Correlation between p53 percentage score and tumor grade

| p53 percentage score | Tumor grade | Cross tabulation | Tumor grade |
|----------------------|-------------|------------------|-------------|
| Negative staining    | 4           | 2                | 6           |
| 5–10% of tumor cells positive for staining (+1) | 4           | 6                | 10          |
| 11–25% of tumor cells positive for staining (+2) | 3           | 4                | 7           |
| 26–50% of tumor cells positive for staining (+3) | 3           | 3                | 6           |
| Over 50% of tumor cells positive for staining (+4) | 0           | 3                | 3           |
| Total                | 14          | 18               | 32          |

\( p = 0.04 \) (significant).

### Table 30: Correlation between pRb intensity score and tumor stage

| pRb intensity score | Tumor stage | Cross tabulation | Tumor stage |
|---------------------|-------------|------------------|-------------|
| Negative staining   | 2           | 8                | 0           |
| Low intensity       | 0           | 8                | 1           |
| Moderate intensity  | 0           | 2                | 1           |
| High intensity      | 0           | 6                | 0           |
| Total               | 1           | 18               | 1           |

\( p = 0.044 \) (significant).

### Table 31: Correlation between pRb percentage score and tumor grade

| pRb percentage score | Tumor stage | Cross tabulation | Tumor stage |
|----------------------|-------------|------------------|-------------|
| Negative 0%          | 0           | 2                | 0           |
| 1–25% (+1)           | 0           | 9                | 3           |
| 26–50% (+2)          | 0           | 4                | 3           |
| 51–75% (+3)          | 1           | 3                | 0           |
| Total                | 1           | 18               | 1           |

\( p = 0.042 \) (significant).

### Correlations between p53 scores and tumor stage

Between p53 intensity score and tumor stage

The difference between different tumor stages regarding p53 intensity scores was significant \( p = 0.018 \) as shown in Table 32.

### Table 32: Correlation between p53 intensity score and tumor stage

| p53 intensity score | Tumor stage | Cross tabulation | Tumor stage |
|---------------------|-------------|------------------|-------------|
| Negative staining   | 1           | 3                | 0           |
| Weak or mild staining | 0           | 3                | 2           |
| Moderate staining   | 0           | 4                | 4           |
| Strong staining     | 0           | 0                | 6           |
| Total               | 1           | 18               | 1           |

\( p = 0.018 \) (significant).

### Table 33: Correlation between p53 percentage score and tumor stage

| p53 percentage score | Tumor stage | Cross tabulation | Tumor stage |
|----------------------|-------------|------------------|-------------|
| Negative for staining | 1           | 3                | 0           |
| 5–10% of tumor cells positive for staining (+1) | 0           | 5                | 0           |
| <25% of tumor cells positive for staining (+2) | 0           | 3                | 2           |
| 25–50% of tumor cells positive for staining (+3) | 0           | 5                | 1           |
| Over 50% of tumor cells positive for staining (+4) | 2           | 0                | 0           |
| Total                | 1           | 18               | 6           |

\( p = 0.019 \) (significant).
Discussion

There is a progressive worldwide increase in the incidence and death rates from malignancy over the world including urinary bladder carcinoma which is regarded as one of the commonest ten cancers in Iraq [21]. BLCA is a worldwide problem, and the second most common malignancy of the genitourinary system [22]. Ninety percent of BLCAs are superficial in nature and urothelial TCC accounts for approximately 90% of them [23]. The molecular phenotyping has shown a new dimension to the characterization of the biological potential of the tumors which may help in better prediction of their clinical outcome. Different studies have revealed that alteration in cell cycle regulation is a major key event in determining the biological behavior of bladder carcinoma [24]. The P53 gene is a tumor suppressor gene playing an essential role in regulation of the cell cycle. So that, when DNA damage occurs, the level of P53 protein is increased leading to cell cycle arrest and repair of the damaged DNA. Mutations in the P53 gene will result in the production of abnormal protein products, allowing cells with damaged DNA to continue through the cell cycle [25]. The Rb gene (RB) mutation is responsible for the Rb; however surviving patients are particularly prone to develop a second primary tumor, particularly osteosarcoma, small cell lung carcinoma, soft tissue sarcomas, breast carcinoma, and genitourinary carcinomas [26].

In this study, the age ranged from 28 to 87 years with mean age (60.2) years, which was not came in accordance with data from other Iraqi studies which had reported a different mean age by Batool [23] with mean age (56.8) years, while the study of Mazin [27] was corresponding to this study with mean age (60) years. In this study, the percentage of male to female
distribution was (2.2:1), which is rather less than the ratio mentioned by Velthoven et al. [28]. It is comparable to other studies in our country like these recorded by Mazin [27] and Batool [23] (2.75/1, and 2.69/1 respectively) and to Neal et al. study that showed incidence rates of 144.0/100,000 person-years in men and 34.5/100,000 person-years in women [29]. The male preponderance is related to social, cultural and religious consideration of female patients in addition to that female confined more to house activities, while male is the main field worker. According to Neal et al., cigarette smoking is a strong risk factor for bladder carcinoma in both males and females. Compared to never-smokers (69.8/100,000 person-years in males and 16.1/100,000 person-years in females), x smokers and current smokers have an increased risk of BLCA in both males (x smokers, 154.6/100,000 person-years and current smokers, 276.4/100,000 person-years) and females (x smokers, 40.7/100,000 person-years; and current smokers, 73.6/100,000 person-years) [29]. In this study, the percentage of smokers was more than non-smokers (59.4 vs. 40.6%), while it was (70% vs. 30%) as recorded by Mazin [27] and (68.7% vs. 31.2%) as recorded by Batool [23]. Chronic bacterial infection with urinary calculus and obstruction may predispose to development of BLCA. Squamous cell carcinoma is the most common entity in these cases. Compared to non-squamous carcinoma, schistosomiasis is more commonly associated with squamous cell carcinoma [30]. In this study (14) (43.8%) out of 32 patients with TCC had UTI with 55.5% of the high grade tumors have history of UTI. The positive relationship between history of recurrent lower UTI was showed in a study of Vermeulen et al. that revealed regular lower UTI is associated with increased risk of urinary BLCA (men: 6.6 [4.2–11]; women: 2.7 [2.0–3.5]), with much stronger effects in muscle-invasive cancers [31]. In this study, (5) (15.6%) out of 32 patients with TCC had renal stones and this is less what had been recorded in other study done in Brazil by Tobias-Machado et al. [32] (33%) of the patients had renal stones, this difference might due to different time and place. In the epidemiologic studies, positive family history carries a two-fold increase in the BLCA risk, however, it is uncertain whether this is due to a genetic and/or the shared environmental factors for familial aggregation [33]. A new study demonstrated that hereditary non-polyposis colorectal cancer was associated with an increased risk of BLCA. Since 2008, genome-wide association study (GWAS) had been used to identify the susceptibility loci for bladder carcinoma [34]. In this study, (3) (9.4%) out of 32 patients had positive family history for TCC. In this study, (17) (53.1%) out of the 32 patients with TCC presented with recurrent tumor and this came in accordance with another much larger study done by Hall et al. [35] in Dallas USA which shows (51%) out of 252 patients with TCC had recurrence. However, this was almost the double the percentage shown by study done in our country by Al-Abbasi [36] who shows that 29.63% out of 54 patients with TCC had recurrent tumor. These differences could be explained the selection criteria and sample size. In this study, 14 (43.8%) out of 32 patients were with low grade TCC while 18 (56.2%) were with high grade TCC which disagree with other study done by Kadhim et al. [37] there sample where include in the region of Middle East (Jordan, Syria and Iraq) who found that 66% of cases where with high grade tumor versus 33% where of low grade tumor. Probably, different environmental and ethnic factors operate in these three countries. In this study (24) (75%) out of 32 patients with TCC was in T1 category of tumor size and stage and this was much less as recorded by study done by Al-Abbassi [36] who shows (18.51%) patients out of 54 patients with TCC which lie in this category but in other study done in USA by Cheng et al. [38] who shows that (52%) out of 105 patients with TCC lie in this category. In this study, (3.1%) 1 patient out of 32 TCC patients shows regional lymph node metastasis while it was (15.9%) 21 patients out of 132 TCC patients in a study done by Nakanishi et al. [39] in Japan. These differences can be attributed to differences in the sample size and criteria of patient selection. In this study there was no statistical significant association between age and tumor grade since \( p = 0.1 \), several studies support our conclusion such as Iranian study done in Sina hospital by Mohseni et al. [40] (\( p = 0.59 \)), also Yang et al. have concluded the same results and noted that patient’s age is not a significant predictor for the prognosis [41]. Yossepowitch and Dalbagni [42] also found that there was no difference in the pathological grade distribution in young adults as compared to the older individuals. However, a study done in USA by Hall et al. showed that there was an association between tumor grade and age and the \( p \) value was (0.042) [35]. Our study showed a higher incidence of both high and low grade TCC in males. There was no correlation between gender of the patients in this study and tumor grade (\( p = 0.288 \)). However, an Iranian study conducted by Mohseni et al. [40] showed an association between gender and tumor grade (\( p = 0.029 \)). Similarly, Batool also showed a higher incidence of high grade tumor in females. However, it came in correspondence to study done by Sunita et al. [43]. These differences are probably related to sample size and patients selection. In our study, smoking showed no correlation with the tumor grade (\( p = 0.275 \)). Other studies, however, found a positive association between these two parameters for example, the study conducted by Mohseni et al. [40]. The deviation of our results from these and other studies is certainly related to the smaller sample size. There was a strong association between tumor grade and stage in our study \( p = 0.003 \) and this is like what revealed in the study done by Hall et al. [35] \( p = 0.0001 \) and in other study done by Cheng et al. [38] \( p = 0.002 \) and also this shown by study done Edinburgh, UK by Stewart et al. [44] \( p = 0.001 \). Regarding pRb scores, two cases
Yahya et al. Immunohistochemical Expression of Retinoblastoma Gene Product and p53 Protein in Transitional Cell Carcinoma (6.2%) out of the presented 32 patients with TCC with negative pRb intensity score and two patients with negative percentage score. The remaining 30 patients show different intensity and percentage scores. In this study, 16 patients (50%) with low score, 11 patients (34.4%) revealed moderate intensity, and three patients (9.2%) revealed high intensity. While 15 patients (46.9%) revealed +1 score, 11 patients (34.4%) revealed +2 score, and four patients (12.5%) revealed +3 score. While Wright et al. [45] found that Rb protein was undetectable in (18%) out of 84 patients with TCC of the bladder, Cordon-Cardo et al. [46] scored (19%) as negative for Rb expression, although their negative group also included tumor showing <10% positive cells. Regarding the P53 scores, there was a significant overexpression of P53 among the studied 32 TCCs with a positive frequency of 82.2% (p < 0.0001). Similar results have been quoted by many investigators such as Du et al. [47], and Al-Abassi [36]; their positive frequencies were 82% and 83.33%, respectively, with a (p < 0.0001). However, other investigators cited lower frequency figures of P53 positivity such as Sarks et al. [48], Lu et al. [49] and Sunanda et al. [24]; their quoted positive frequencies were 58%, 50.7%, and 62%, respectively. The difference between low-grade and high-grade tumors regarding pRb percentage score was statistically significant (p = 0.026), but it was not significant regarding the intensity score (p = 0.094). Shariat et al. [50] and Khaled [51] found that altered Rb protein expression was not associated with tumor grade (p = 0.622 and 0.71, respectively). In this study, there was significant correlations between tumor stage and both pRb intensity and percentage scores (p = 0.044 and 0.042, respectively). Similar results were obtained by Shariat et al. [50] who found that altered Rb protein expression is associated with different tumor stages and the degree of tumor invasion (p = 0.003). Along the same lines, Khaled [51] found significant correlation between tumor stage and Rb protein expression (p = 0.023).

The difference between low grade and high grad regarding p53 intensity score was significant p = 0.022. In agreement with these findings, Shihina et al., [52] found that p53 is positively correlated with histological grade of tumor also Al-Abassi [36] found it correlated with P = 0.003. The difference between low grade and high grade regarding p53 percentage score was significant p = 0.049. In agreement with our study, many investigators reported that p53 nuclear immunostaining was associated with the grade of BLCA. Cheng et al., [53] showing that p53 immunostaining was positive in 26% of Grade I, 57% of grade II, and 42% of grade III. Findings of the current work also agreed by Kilicli-Camur et al. [54], Lu et al. [49], and Sunanda et al. [24]. Al-Abassi [36] also found it correlated with p = 0.013. The difference between different tumor stages regarding p53 intensity score was significant and p = 0.018. From these readings of P53 overexpression, it looks that as the size of tumor increases, more P53 immunostaining will be noticed. In agreement with the current study by Kilicli-Camur et al., [54], while Cheng et al. [53] reported that p53 immunostaining was positive in 23% of Ta-T1, 15% of T2-3, and 15% of T4. The difference between different tumor stages regarding p53 percentage score was significant p = 0.019. In agreement with these findings were reported by Al-Abassi [36] with p = 0.014. Also this reported by Lu et al., [50], and Kilicli-Camur et al., [54]. In the current work and in comparison between stage Ta and stage T3 and T4, a significant difference was found (p < 0.05). This finding is in agreement with studies reported by Kilicli-Camur et al. [54] and Liuxi et al. [55] who found that p53 mutation with low expression of PCDH17 was significantly associated with MIBC. P53 was found to be more frequently expressed in those with advanced stage that explains the aggressive biological behavior of tumor, which is well known to be correlated with the degree of differentiation.

**Conclusions**

Tumor’s grade was found to be correlated with the tumor stage but not with the patient’s age, gender, family history of TCC, smoking habits, history of urinary tract infections nor lithiasis, nor the recurrence of the tumor. The pRb intensity and the percentage scores were correlated to each other and to tumor’s grade and stage, except for the pRb intensity which showed no correlation with the tumor’s grade. The P53 intensity and percentage scores were correlated to each other and also to tumor’s grade and stage, so that P53 is over expressed in tumors with higher grade and stage.

**References**

1. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer statistics, 2005. CA Cancer J Clin. 2005;55(1):10-30. https://doi.org/10.3322/canjclin.55.1.10

2. Reddy PV. Prognostic value of P53 nuclear overexpression in bladder cancer. Int J Med Res Rev. 2017;5(6):569-70.

3. Crow P, Ritchie AW. National and international variation in the registration of bladder cancer. BJU Int. 2003;92(6):563-6. https://doi.org/10.1046/j.1464-410x.2003.04421.x

4. Pernick N. p53. Available from: https://www.pathologyoutlines.com/topic/stainsp53.html. [Last accessed on 2021 Mar 13].

5. Goussia AC, Alexandra PB, Antonia C, Panagiotits K, Panagiotis K, John KE, et al. Alterations of p53 and Rb pathways are associated with high proliferation in bladder urothelial carcinomas. Anticancer Res. 2018;38(7):3985-88. https://doi.org/10.21873/anticanres.12685

PMid:29970521
6. Malumbres M, Barbadid M. To cycle or not to cycle: A critical decision in cancer. Nat Rev Cancer. 2001;1(3):222-31. https://doi.org/10.1038/35106065 PMid:11902577

7. Marr BP, Hung C, Gobin YP, Dunkel IJ, Brodie SE, Abramson DH. Success of intra-arterial chemotherapy (chemosurgery) for retinoblastoma: Effect of orbitovascular anatomy. Arch Ophthalmol.. 2012;130(2):180-5. https://doi.org/10.1001/archophthalmol.2011.398 PMid:22332209

8. Ghassemi F, Shields CL. Intravitreal melphalan for refractory or recurrent vitreous seeding from retinoblastoma. Arch Ophthalmol.. 2012;130(10):1268-71. https://doi.org/10.1001/archophthalmol.2012.1983 PMid:23044940

9. Munier FL, Gaillard MC, Balmer A, Soliman S, Podlisly G, Moulin AP, et al. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: From prohibition to conditional indications. Br J Ophthalmol.. 2012;96(8):1078-83. https://doi.org/10.1136/bjophthalmol-2011-301450 PMid:22694968

10. Liukkonen T, Lipponen P, Raitanen M, Kaasiner E, Ala-Kairasheva MV, et al. Cyclin D1 expression in papillary superficial bladder cancer: Towards clinical application. Nat Rev Urol. 2015;12(6):317-30. https://doi.org/10.1038/nrurol.2015.100 PMid:26032553

11. Sgambato A, Migaldi M, Faraglia B, De Aloysio G, Ferrari P, Ardito R, et al. Cyclin D1 expression in papillary superficial bladder cancer. Clin Cancer Res. 2009;15(9):3092-7. https://doi.org/10.1158/1078-0432.CCR-08-2671 PMid:19530097

12. Tsai TS, Tsai YS, Chow NH. The prevalence and clinicopathologic correlate of p16INK4a, retinoblastoma and p53 immunoreactivity in locally advanced urinary bladder cancer. J Urol. 2000;164(6):2134-37. https://doi.org/10.1016/s0022-5347(05)66984-4 PMid:11061942

13. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Meeks G, et al. Intravitreal chemotherapy for vitreous disease in retinoblastoma revisited: From prohibition to conditional indications. Br J Ophthalmol.. 2012;96(8):1078-83. https://doi.org/10.1136/bjophthalmol-2011-301450 PMid:22694968

14. Mak RH, Hunt D, Shipey WU, Efstrathiou JA, Tester WJ, Hagan MP, et al. Long-term outcomes in patients with muscle-invasive bladder cancer after selective bladder-preserving combined-modality therapy: A pooled analysis of radiation therapy oncology group protocols 8802, 8903, 9506, 9706, 9906, and 0233. J Clin Oncol. 2014;32(34):3801-9. https://doi.org/10.1001/jco.2014.57.5548 PMid:25366878

15. Woldu SL, Bagrodia A, Lotan Y. Guideline of guidelines: Non-muscle-invasive bladder cancer. BJU Int. 2017;119(3):371-80. https://doi.org/10.1111/bju.13780 PMid:28058776

16. Amin MB, Smith SC, Reuter VE, Epstein JB, Grignon DJ, Hansel DE, et al. Guideline of guidelines: Non-muscle-invasive bladder cancer. BJU Int. 2015;28:612-30.

17. Mitra AP. Molecular substratification of bladder cancer: Moving towards individualized patient management. Ther Adv Urol. 2016;8(3):215-33. PMid:27247631

18. Frantz M, Latosinska A, Flueh L, Hupe M, Crisilesis E, Kramer MW, et al. Developing proteomic biomarkers for bladder cancer: Towards clinical application. Nat Rev Urol. 2015;12(6):317-30. https://doi.org/10.1038/nrurol.2015.100 PMid:26032553

19. Blancato J, Singh B, Liu A, Liao DJ, Dickson RB. Correlation of amplification and overexpression of the c-myc oncogene in high grade breast cancer: FISH, in situ hybridization and immunohistochemical analysis. Br J Cancer. 2004;90(8):1612-19. https://doi.org/10.1038/sj.bjc.6601703 PMid:15083194

20. Apple SK, Hecht JR, David W. Immunohistochemical evaluation of K-ras, P53, and HER-2/neu expression in hyperplastic, dysplastic, and carcinomatous lesions of the pancreas: Evidence for multistep carcinogenesis. Hum Pathol. 1999;30(2):123-30. https://doi.org/10.1016/s0046-8177(99)02654-5 PMid:10029438

21. Iraqi Cancer Registry 2012/Ministry of health/Iraq. Available from: https://www.bccru.uobaghdad.edu.iq. [Last accessed on 2021 Mar 14].

22. Daniel L. TNM staging: The common language for cancer care. Am Joint Committee Cancer. 2008.

23. Batool JJ, Al-heety: Immunohistochemical expression of caspase-3 and Bcl-2 in urinary bladder carcinoma. J Urol. 2010;4(5):42-74.

24. Chatterjee SJ, Datar R, Youssefzadeh D, George B, Goebell PJ, Stein JP, et al. Combined effects of p53, p21, and pRb expression in the progression of bladder transitional cell carcinoma. J Clin Oncol. 2004;22(6):1007-13. https://doi.org/10.1200/jco.2004.05.174 PMid:14981105

25. Lichtenstein AV, Potapova GI. Genetic defects as tumor markers. J Mol Biol. 2003;37(2):159-69.

26. Favoni RE, De Cupis A. The role of polypeptide growth factors in human carcinomas: New targets for a novel pharmacological approach. Natl Cancer Inst. 2000;52(2):179-206. PMid:10835099

27. Mazin J. Clinicopathological study of urinary bladder carcinoma by detection of the molecular marker p53 by in situ hybridization, before and after treatment. 2009;4:63-74.

28. Velthoven RV, Petie RM, Osterlink W, Kiss R, Decaestecker C. Identification by quantitative chromatin pattern analysis of patients at risk for recurrence of superficial transitional bladder carcinoma. J Urol. 2000;164(6):2134-37. https://doi.org/10.1016/s0022-5347(05)66984-4 PMid:11061942

29. Freedman ND, Silverman DT, Hollenbeck AR, Schatzkin A, Adami HO, et al. Association between smoking and risk of bladder cancer among men and women. JAMA. 2011;306(7):737-45. https://doi.org/10.1001/jama.2011.1142 PMid:21468655

30. Rambau PF, Chalya PL, Jackson K. Schistosomiasis and urinary bladder cancer in North Western Tanzania: A retrospective review of 185 patients. Infect Agent Cancer. 2013;8(1):19. https://doi.org/10.1186/1750-9378-8-19 PMid:23705833

31. Vermeulen SH, Hanum N, Grotenhuis AJ, Castaño-Víyals G, van der Heijden AG, Aben KK, et al. Recurrent urinary tract infection and risk of bladder cancer in the Nijmegen bladder cancer study. Br J Cancer. 2015;112(3):594-600. https://doi.org/10.1038/bjc.2014.601 PMid:25429525

32. Machado MT, Pinto MA, Juliano RV, Mattos MH, Wroclawski ER. Alternatives for distal ureter resection in laparoscopic nephroureterectomy. Braz J Urol. 2002;28(2):109-15.

33. Mueller CM, Caporaso N, Greene MH. Familial and genetic risk of transitional cell carcinomas of the urinary tract.
Yahya et al. Immunohistochemical Expression of Retinoblastoma Gene Product and p53 Protein in Transitional Cell Carcinoma

Urol Oncol. 2008;26(5):451-64. https://doi.org/10.1016/j.urolonc.2008.02.016

34. Chu H, Wang M, Zhang Z. Bladder cancer epidemiology and genetic susceptibility. J Biomed Res. 2013 May; 27(3):170-8. PMid:18652223

35. Hall MC, Womack S, Sagalowitz Al, Carmody T, Erickstad MD, Roehrborn CG. Prognostic factors, recurrence, and survival in transitional cell carcinoma of the upper urinary tract: A 30-year experience in 252 patients. Urology. 1998;52(4):594-601. https://doi.org/10.1016/s0090-4295(98)00295-7 PMid:9763077

36. Al-Abassi D. IHC study of TCC of urinary bladder by application of VEGF in correlation to P53. BMC Res Notes. 2008;3:31-54.

37. Kadhim HS, Abdulamir AS, Hafith RR, Abbas KA. Investigations in the molecular events of transitional cell carcinoma of the bladder. Am J Biochem Biotechnol. 2008;4(4):408-15. https://doi.org/10.3844/ajbbsp.2008.408.415

38. Cheng L, Neumann RM, Weaver AL, Cheville JC, Leibovich BC, Ramnani DM, Scherer BG, et al. Grading and staging of bladder carcinoma in transurethral resection specimens. Am J Clin Pathol. 2000;113(2):275-79. https://doi.org/10.1093/ajcp/113.2.275 PMid:10664630

39. Nakanishi K, Kawai T, Aida S, Kasamatsu H, Auruess T, Ikeda T. Expression of p27 protein in transitional cell carcinoma of the upper urinary tract. Mod Pathol. 2001;14(5):371-76. PMid:11353044

40. Mohseni MG, Nourbakhsh A, Hatami ZN. Association of smoking with high-grade transitional cell carcinoma of the urinary bladder. Arch Iranian Med. 2005;8(4):286-9. https://doi.org/10.1038/modpathol.3880320

41. Yang MH, Yen CC, Chen PM, Wang WS, Chang YH, Huang WJ, Aurues T, Ikeda T. Overexpression of c-met as a prognostic indicator for transitional cell carcinoma of bladder: A marker for disease progression. J Natl Cancer Inst. 1999;85(1):53-9. https://doi.org/10.1093/jnci/85.1.53 PMid:7677935

42. Lu ML, Wikman F, Omtlof TF, Charytonowicz E, Rabbani Farhang, Zhang Z, et al. Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome. Clin Cancer Res. 2002;8(1):171-9. PMid:11801555

43. Shariat SF, Takunaga H, Zhou JH, Kim JH, Ayala GE, Benedict WF, et al. P53, p21, pRb, and p16 Expression predict clinical outcome in cystectomy with bladder cancer. J Clin Oncol. 2004;22(6):1014-24. https://doi.org/10.1200/jco.2004.03.118 PMid:14981102

44. Khaled HM, Bahnassy AA, Raafat AA, Zekri AN, Madboul MS, Mokhtar NM. Clinical significance of altered nm23-H1, EGFR, RB and p53 expression in bilharzial bladder cancer. BMC Cancer. 2009;9:32. https://doi.org/10.1186/1471-2407-9-32 PMid:19171060

45. Shina H, Igawa M, Shigeno K. Clinical significance of mdm2 and p53 expression in bladder cancer. A comparison with cell proliferation and apoptosis. Oncology. 1999;56(3):239-47. https://doi.org/10.1055/s-2000-25532 PMid:10202280

46. Cheng HL, Trink B, Tzai TS, Liu HS, Chan SH, Ho CL, et al. Overexpression of c-met as a prognostic indicator for transitional cell carcinoma of the urinary bladder: A comparison with p53 nuclear accumulation. J Clin Oncol. 2002;20(6):1544-50. https://doi.org/10.1200/jco.2002.20.6.1544 PMid:11896103

47. Camur NK, Kilicaslan I, Gulluoglu MG, Esen T, Uysal V. Impact of p53 and Ki-67 in predicting recurrence and progression of superficial (pTa and pT1) urothelial cell carcinomas of urinary bladder. Pathol Int. 2002;52(7):463-69. https://doi.org/10.1046/j.1440-1827.2002.01371.x PMid:12167105

48. Saxena S, Burra U, Varma S, Aggarwal A, Tripathi MJ. Role of in vitro Cytotoxicity Assessment and Immunologic Enhancement in the Management of Superficial Bladder Cancer. 2004;p20-22.

49. Wright C, Thomas D, Mellon K, Neal DE, Horne CH. Expression of retinoblastoma gene products and p53 protein in bladder carcinoma: Correlation with ki67 index. Br J Urol. 1995;75(2):173-9. https://doi.org/10.1111/j.1464-410x.1995.tb07306.x PMid:8750321

50. Cordon-Cardo C. Mutation of cell cycle regulators: Biological and clinical implications for human Neoplasia. Am J Pathol. 1995;147(3):545-60. PMid:7677168

51. Du J, Chen GG, Vlantis AC, Xu H, Tsang RK, van Hasselt AC. P53 in predicting recurrence and progression of urothelial cell carcinomas of urinary bladder. Korean J Urol. 2003;44:256-61.

52. Sarkis AS, Dalbagni G, Cordon-Cardo G, Zhang ZF, Sheinfeld J, Fair WR, et al. Nuclear overexpression of p53 protein in transitional cell carcinoma of bladder: A marker for disease progression. J Natl Cancer Inst. 1993;85(1):53-9. https://doi.org/10.1093/jnci/85.1.53

53. Saxena S, Burra U, Varma S, Aggarwal A, Tripathi MJ. Role of in vitro Cytotoxicity Assessment and Immunologic Enhancement in the Management of Superficial Bladder Cancer. 2004;p20-22.

54. Camur NK, Kilicaslan I, Gulluoglu MG, Esen T, Uysal V. Impact of p53 and Ki-67 in predicting recurrence and progression of superficial (pTa and pT1) urothelial cell carcinomas of urinary bladder. Pathol Int. 2002;52(7):463-69. https://doi.org/10.1046/j.1440-1827.2002.01371.x PMid:12167105

55. Liuxi C, Ying L, Qi Z, Mingming Z, Xuemeng H, Qiujie L, et al. Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome. Clin Cancer Res. 2002;8(1):171-9. PMid:11801555

56. Shariat SF, Takunaga H, Zhou JH, Kim JH, Ayala GE, Benedict WF, et al. P53, p21, pRb, and p16 Expression predict clinical outcome in cystectomy with bladder cancer. J Clin Oncol. 2004;22(6):1014-24. https://doi.org/10.1200/jco.2004.03.118 PMid:14981102

57. Khaled HM, Bahnassy AA, Raafat AA, Zekri AN, Madboul MS, Mokhtar NM. Clinical significance of altered nm23-H1, EGFR, RB and p53 expression in bilharzial bladder cancer. BMC Cancer. 2009;9:32. https://doi.org/10.1186/1471-2407-9-32 PMid:19171060

58. Shina H, Igawa M, Shigeno K. Clinical significance of mdm2 and p53 expression in bladder cancer. A comparison with cell proliferation and apoptosis. Oncology. 1999;56(3):239-47. https://doi.org/10.1055/s-2000-25532 PMid:10202280

59. Cheng HL, Trink B, Tzai TS, Liu HS, Chan SH, Ho CL, et al. Overexpression of c-met as a prognostic indicator for transitional cell carcinoma of the urinary bladder: A comparison with p53 nuclear accumulation. J Clin Oncol. 2002;20(6):1544-50. https://doi.org/10.1200/jco.2002.20.6.1544 PMid:11896103

60. Camur NK, Kilicaslan I, Gulluoglu MG, Esen T, Uysal V. Impact of p53 and Ki-67 in predicting recurrence and progression of superficial (pTa and pT1) urothelial cell carcinomas of urinary bladder. Pathol Int. 2002;52(7):463-69. https://doi.org/10.1046/j.1440-1827.2002.01371.x PMid:12167105

61. Liuxi C, Ying L, Qi Z, Mingming Z, Xuemeng H, Qiujie L, et al. Impact of alterations affecting the p53 pathway in bladder cancer on clinical outcome. Clin Cancer Res. 2002;8(1):171-9. PMid:11801555