International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

# Significance of Her2/Neu, C-kit and P63 Immunohistochemical Expression in Bladder Urothelial Carcinoma

**Mai M. Abdelwahab[1], Samah S. Elbasateeny[2]**

Pathology Department, Faculty of Medicine, Zagazig University, Egypt

**Abstract:** ***Background: Urothelial carcinoma (UC) is a devastating disease with high mortality rate. Patients at same grade/ stage can have different outcomes, which necessitate better prognostic markers for estimating the risk of progression. Aim: To assess the impact of Her2 /Neu, C-kit, and P63 on carcinogenesis and behavior of UC. Methods: Her2/Neu, C-kit, and P63 were assessed by immunohistochemistry in 60 cases of UC, and correlated with clinicopathological parameters. Results: Her2/Neu was expressed in 40% of cases. It showed positive expression in 33.3% of low grade and 44.4% of high grade UC. It was expressed in 14.3%, 47.8% of non-invasive and invasive UC respectively, with significant association with grade and stage (p=0.023, <0.001 respectively). C-kit was expressed in 46.7% of cases with significant association with both grade and stage (p <0.00). P63 was expressed in all low grade and in 66.7% of high-grade UC cases, it was expressed in all non-invasive and in 73.3% of invasive cases with significant association with grade and stage (p =0.001, 0.001 respectively) (decreased expression with increasing grade /stage). A significant positive correlation was detected between Her2/Neu, C-kit ( τ =+0.35 ,p=0.001), while a significant strong inverse correlation was observed between P63 and both Her2/Neu (τ =-0.641 ,p<0.001), and C-kit (τ = -0.558 ,p<0.001). Conclusion: Her2\Neu, C-kit overexpression and loss of P63 are important players in carcinogenesis of UC and could be considered as poor prognostic parameters. Patients must be examined for Her2/Neu and C-kit status for selection of suitable candidates for adjuvant targeted therapies.***

**Keywords:** Urothelial carcinoma, Immunohistochemistry, Her2 /Neu, C-kit, P63

## 1. Introduction

Urothelial carcinoma (UC) is a devastating disease with high mortality rate. It is the commonest malignancy of the urinary tract, representing the seventh leading cause of cancer related death worldwide [1].

Among Egyptian men, bladder cancer is the commonest cancer, and it has mortality 4 folds higher than the rates in USA and 2 folds higher than the rates in Europe [2]. Urothelial carcinoma is the commonest type of urinary bladder malignancy, accounting for more than 90% of all patients with bladder cancer [3]**.** In Egypt, there was a considerable increase of UC from 16% up to 66%, to become the commonest tumor type nowadays, with a considerable reduction in squamous cell carcinoma (SCC) from 76 % to about 28% [4]**.** This may be due to increased exposure to etiological factors as smoking and pesticides [5]. The management of UC patients depends mainly on the clinico-pathological parameters, such as grading and staging of the neoplasm, as indicators of the outcome. Though, these factors are not sufficient to expect the prognosis of the patients and show marked divergences within the same tumor grade or stage. This is mostly due to marked heterogeneity of the cells of bladder malignancy [6]**.** Many researches are being carried out to develop new molecular methods that can help in the diagngnosis of the tumor at an initial stage, improve outcome of high risk patients and clinical managing [7]. Although at first hopeful, the majority of these methods are not adequately sensitive or specific which require the development of further molecular markers that are more sensitive in expecting patient's outcome and consequently improving the surveillance of bladder cancer in clinical setting [8].

Human epidermal growth factor receptor 2 (HER2), is a type-I trans-membrane growth factor receptor. It is placed on chromosome 17q21, and plays a significant role in cellular divisions and tumorigenesis [9]. The role of Her 2 /Neu has been most studied in cancer breast, in which active Her 2 /Neu is overexpressed in 18-22% of cases, correlating with poor prognosis [10]. Overexpression of Her 2 /Neu has been noticed in numerous malignancies, such as lung malignancy [11]**,** ovarian cancer [12]**,** colon cancer [13] and salivary gland cancer [14].

However, the prognostic value of Her2/Neu status in UC remains uncertain. Several studies showed that higher Her2 /Neu expression levels are associated with poor prognosis [15, 16]**.** In contrast, other analysis showed only limited or no prognostic significance of Her2/Neu expression [17,18,19]. The C-kit is a proto-oncogene encoding a 145-160 KDA, type III transmembrane tyrosine kinase receptor named as c-KIT or CD 117 [20]. Appearance of C-kit is important in the growth of some kinds of cells, such as germ cells, interstitial cells of Cajal, erythrocytes, and mast cell [21]**.** Amplifications of C-kit has been noticed in numerous malignancies, such as gastrointestinal stromal tumor, small cell carcinoma of the lung and carcinoma of breast,. However, neoplastic development or progression of bladder cells may express cell surface proteins and activation of kinases [22].

P63 is a transcriptional factor that belongs to the family of p53 and shares structural homology with p53. The gene encoding P36 protein is localized on chromosome 3q27-29 [23]**.** P63 is a marker of basal epithelial cells that is essential for normal growth of numerous epithelial tissues such as bladder and prostatic tissues [24]. Different researches enrich the theory that P63 can work as a tumor suppressor as its amplification is accounting for stimulation of P53

responsive genes resulting in cell cycle stoppage and apoptosis. Furthermore, it could also mediate apoptosis by activating the complexes of death receptor (CD95, TRAIL) and the death pathway of the mitochondria (BAX, APAF1) [25].

Impaired P63 expression is thought to be a prognostic marker along with the well-established prognostic factors, such as TNM stage [26]. In this work, we aimed to assess the expression of her2 /Neu, C-kit, and P63 in UC and correlate their immunohistochemical expressions with the pathological parameters to identify their impact on tumor behavior and carcinogenesis.

## 2. Material and Methods

**Material**

The specimens of the current study were obtained from UC cases admitted to The Urology Department and referred to the Pathology Laboratory at Zagazig University hospital, in the period between May 2015 and June 2016.The total number of the studied cases was 60 , divided into 24 cases low-grade UC, 36 cases high-grade UC, 14 non-invasive UC and 46 invasive UC. Clinical data were obtained from the referral clinical reports. The biopsies were obtained by either transurethral resection (TUR)(45 cases) or radical cystectomy (15 cases). Each case in the study was stained by routine H&E stain to evaluate the diagnosis. The grading and staging were assessed following the tumor classification of World Health Organization 2004[27] and American Joint Committee on Cancer 2010[28], respectively.

**Immunohistochemistry**

The immunohistochemical staining procedure was performed according to streptavidin–biotin immunoperoxidase method (Dako-Cytomation, Glostrup, Denmark). Sections were cut in 3–5 µm thickness from the formalin-fixed-paraffin-embedded blocks, put on positively charged slides followed by removal of the paraffine with aid of xylene, and rehydrated using graded alcohol. Thereafter, tissue sections were heated in buffered citrate (pH 6.0) for about 20 minutes, and then washed in PBS (pH 7.3). After that, endogenous peroxidase activity was stopped with 6% H2O2 in methanol. Then, the slides were incubated overnight with mouse monoclonal antibodies against Her2/Neu (Clone e2-4001,catalog no.MS-730-R7,Lab vision, California,USA, ready to use) , monoclonal antibody against C-kit (Clone 104D2, Dako Denmark, 1:200) and a mouse monoclonal P63 antibody (Clone 4A4, catalog # (CM163A, B, C, H. BIOCARE MEDICAI, 1:100) .After rinsing in PBS, slides were immersed with a biotin-conjugated secondary antibody (Lab vision Corporation, Fermont, USA). DAB was used as a chromogen and Mayer's hematoxylin as a counter stain, and then the slides were washed with distilled water and PBS. Positive and negative controls were stained with the same setting of the studied cases. The negative controls were done using the same tissue with the omission of the primary antibody.

**Assessment of immunohistochemistry:**

**Her2/Neu immunostaining:** Her2/Neu staining was assesed according to membranous staining pattern and intensity as follow: 0, negative staining or membrane staining in less than 10% of the neoplastic cells; 1+, partial faint membrane staining in more than 10% of the neoplastic cells; 2+, complete weak to moderate staining in more than 10% of the tumor cells; 3+, complete strong membrane staining detected in more than 10% of the neoplastic cells. Score 0 and +1 were regarded as negative, while scores of 2+ and 3+ were regarded as positive for Her 2/Neu[29].

**C-kit immunostaining:** C-kit was expressed heterogeneously as membranous and /or cytoplasmic staining and evaluated depending on the extent and intensity of the staining, as follow: no staining, weak (less than 10% of the selected area); moderate (10%-49% of the selected area) , strong ( intense staining in 50% or more of the selected area). Cases were divided as negative, (The negative and weak) C-kit expression groups, and positive (The moderate and strong) C-kit expression group [30].

**P63 immunostaining:** The tissue sections were inspected by bright field microscope to evaluate the immunostaining positivity by the percentage of positive cells and staining intensity in at least 3 different areas. Positive cells for P63 were recognized by the existence of nuclear staining. Nuclear P63 immunoreactivity was assesed with a 12-point calculated scoring system. First, the percentage of positive cells in each area was scored using a 5-point scale: 0 for <5%, 1 for 5-25%, 2 for 25-50%, 3 for 50-75%, and 4 for over 75%. Second, the intensity of positive cells was scored using a 3-point scale: 0 for negative, 1 for weak, 2 for moderate, and 3 for strong staining, then, the total score for each area was calculated by multiplying the percentage of positive cells by the intensity of staining score. Finally, the results were grouped as negative (0-1), weak (2-3), moderate (4-6) and strong (7-12) [31].

**Statistics**

Continuous variables were expressed as mean ± SD & median (range), and the categorical variables were expressed as a number (percentage). Continuous variables were checked for normality using Shapiro-Wilk test. To compare between more than two category of non-normally distributed variables, Kruskal Wallis H test was used. Percentage of categorical variables was compared by the use of Pearson's Fisher's exact test or Chi-square test. Trend of change in distribution of relative frequencies between ordinal data were compared using Chi-square test for trend. Strength of relationship between immunohistochemical staining for Her2/Neu, C-kit and P63 were determined by computing Kendall's tau-b correlation coefficient, (+) sign was used as pointer for direct relationship & (-) sign was used as pointer for inverse relationship, also values near to 1 was indicator for strong relationship & values near 0 was indicator for weak relationship. A p-value <0.05 was regarded significant. All statistics were performed by the use of SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA) and MedCalc windows (MedCalc Software bvba 13, Ostend, Belgium).

## 3. Results

**Clinicopathological parameters:**

This study involved 60 specimens of urinary bladder UC, the age range of the studied patients was 44-76 years, mean

age was 62.07±7.46 years, and median age was 61 years. 48/60 (80%) were males and 12/50 (20%) were females. 46 cases were diagnosed as invasive UC and 14 cases were diagnosed as non-invasive UC, 24/60 of cases were low grade and 36 cases were high grade. Tissue specimens were obtained by TUR in 45 cases (75%) and by radical cystectomy in 15 cases (25 %); all were studied for of Her2 /Neu, C-kit, and P63 immunoexpression. The Clinicopathological parameters of our studied cases are summarized in Table 1.

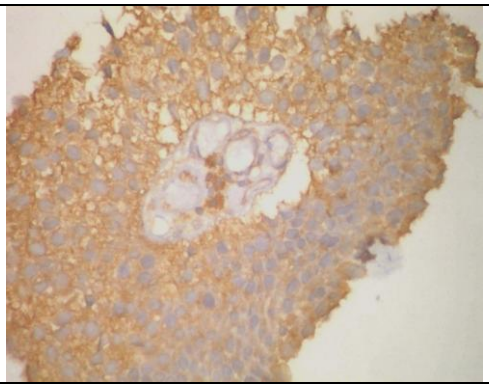
**Table 1:** Clinicopathological features and immunohistochemical staining in 60 patients with bladder urothelial carcinoma

| Characteristics | Number. | Percent |
|---|---|---|
| Age (years) | | |
| Mean ± SD | 62.07 ±7.46 | |
| Median (Range) | 61.00 (44-76) | |
| ≤ 60 years | 28 | 46.7% |
| > 60 years | 32 | 53.3% |
| Sex | | |
| Male | 48 | 80% |
| Female | 12 | 20% |
| Grade | | |
| Low grade | 24 | 40% |
| High grade | 36 | 60% |
| Stage | | |
| Non-invasive (Ta) | 14 | 23.3% |
| Invasive | 46 | 76.7% |
| T1 | 10 | 16.7% |
| T2a | 2 | 3.3% |
| T2b | 14 | 23.3% |
| T3 | 20 | 33.3% |
| Her2/Neu | | |
| Negative | 36 | 60% |
| 0 | 12 | 20% |
| +1 | 24 | 40% |
| Positive | 24 | 40% |
| +2 | 10 | 16.7% |
| +3 | 14 | 23.3% |
| C-kit | | |
| Negative | 32 | 53.3% |
| Negative | 18 | 30% |
| Weak | 14 | 23.3% |
| Positive | 28 | 46.7% |
| Moderate | 12 | 20% |
| Strong | 16 | 26.7% |
| P63 | | |
| Negative | 12 | 20% |
| Weak | 12 | 20% |
| Moderate | 16 | 26.7% |
| Strong | 20 | 33.3% |

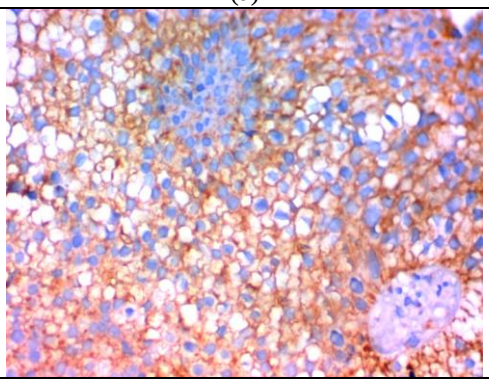
Categorical variables were expressed as number (percentage)
Continuous variables were expressed as mean ± SD & median (range)

## 4. Immunohistochemical Results

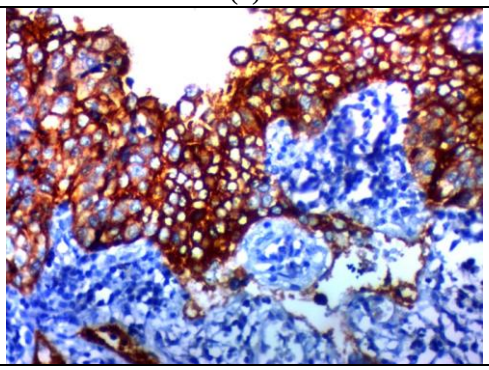
**1- Immunohistochemical expression of Her2/Neu:** Among the 60 cases of UC, 24 cases (40%) expressed positivity for Her2/Neu. There was a significant correlation between Her2/Neu expression and the grade of the tumor (p=0.023), where among low grade tumors, it was overexpressed in 8/24 (33.3%) cases, but negative in 16/24 (66.7%) cases. In high grade UC, it was positive in 16/36 of cases with 33.3% showing score 3 expression. As regarding the stage, Her2/Neu showed positive expression in only 2 cases out of 14 cases of non –invasive UC, while it was positive in 22/46 cases (47.8% ) of invasive UC ,and there was a statistically significant difference between stages (p <0.001). In contrast, patient's age and sex did not show any significant relationship with the expression of Her 2/Neu (p = 0.23, 0.1 respectively) (Figure1, Table 2).



(a)



(b)



(c)

**Figure 1:** ***Immunohistochemical expression of Her2/Neu in bladder urothelial carcinoma:*****(**a) Low grade urothelial carcinoma showing partial faint membranous immunoreactivity for Her2/Neu (Sccore +1).(x200). (b)High grade urothelial carcinoma showing complete moderate membranous immunoreactivity for Her2/Neu (Sccore +2).(x400). (c)High grade urothelial carcinoma showing complete strong membranous immunoreactivity for Her2/Neu (Sccore +3).(x400)

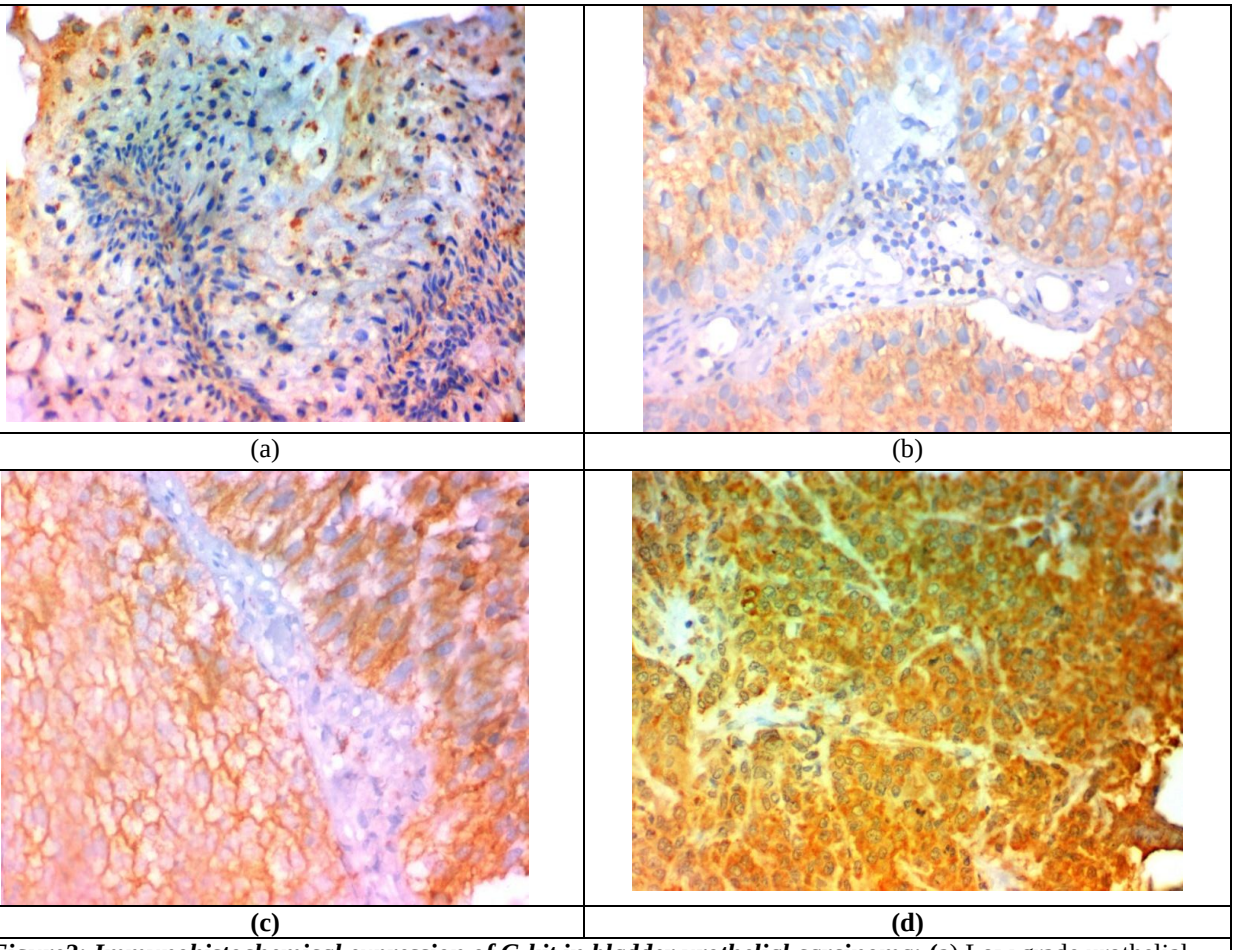
**Table 2:** Relation between clinicopathological features and immunohistochemical expression of Her2/Neu in 60 patients with bladder urothelial carcinoma

| Characteristics | All (N=60) | Her2/Neu | | | | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | Negative (N=36) | 0 (N=12) | +1 (N=24) | Positive (N=24) | +2 (N=10) | +3 (N=14) | |
| | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | |
| Age (years) | | | | | | | | |
| Mean ± SD | 62.1 ±7.46 | 60.8 ±6.61 | 62.5 ±5.74 | 60 ±6.96 | 63.9±8.39 | 62 ±5.69 | 63.1 ±10.03 | 0.079• |
| Median (Range) | 61 (44-76) | 60 (47-75) | 61 (57-74) | 59.5 (47-75) | 63 (44-76) | 64 (59-75) | 62 (44-76) | |
| ≤ 60 years | 28 (46.7%) | 20 (71.4%) | 6 (21.4%) | 14 (50%) | 8 (28.6%) | 2 (7.1%) | 6 (21.4%) | 0.230‡ |
| > 60 years | 32 (53.3%) | 16 (50%) | 6(18.8%) | 10 (31.3%) | 16 (50%) | 8 (25%) | 8 (25%) | |
| Sex | | | | | | | | |
| Male | 48 (80%) | 26 (54.2%) | 10(20.8%) | 16 (33.3%) | 22(45.8) | 8 (16.7%) | 14 (29.2%) | 0.100‡ |
| Female | 12 (20%) | 10 (83.3%) | 2 (16.7%) | 8 (66.7%) | 2 (16.7%) | 2 (16.7%) | 0 (0%) | |
| Grade | | | | | | | | |
| Low grade | 24 (40%) | 16 (66.7%) | 8 (33.3%) | 8 (33.3%) | 8 (33.3%) | 6 (25%) | 2 (8.3%) | 0.023‡ |
| High grade | 36 (60%) | 20 (55.6%) | 4 (11.1%) | 16 (44.4%) | 16 (44.4% | 4 (11.1%) | 12 (33.3%) | |
| Stage | | | | | | | | |
| Non-invasive (Ta) | 14 (23.3%) | 12 (85.7%) | 8 (57.1%) | 4 (28.6%) | 2 (14.3%) | 2 (14.3%) | 0 (0%) | |
| Invasive | 46 (76.7%) | 24 (52.2%) | 4 (8.7%) | 20 (43.5%) | 22(47.8) | 8 (17.4%) | 14 (30.4%) | |
| T1 | 10 (16.7%) | 6 (60%) | 2 (20%) | 4 (40%) | 4 (40%) | 4 (40%) | 0 (0%) | <0.001§ |
| T2a | 2 (3.3%) | 2 (100%) | 0 (0%) | 2 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | |
| T2b | 14 (23.3%) | 8 (57.1%) | 0 (0%) | 8 (57.1%) | 6 (42.9%) | 2 (14.3%) | 4 (28.6%) | |
| T3 | 20 (33.3%) | 8 (40%) | 2 (10%) | 6 (30%) | 12 (60%) | 2 (10%) | 10 (50%) | |
| C-kit | | | | | | | | |
| Negative | 32 (53.3%) | 24 (75%) | 8 (25%) | 16 (50%) | 8 (25%) | 6 (18.8%) | 2 (6.3%) | |
| Negative | 18 (30%) | 12 (66.7%) | 6 (33.3%) | 6 (33.3%) | 6 (33.3%) | 4 (22.2%) | 2 (11.1%) | |
| Weak | 14 (23.3%) | 12 (85.7%) | 2 (14.3%) | 10 (71.4%) | 2 (14.3%) | 2 (14.3%) | 0 (0%) | 0.001§ |
| Positive | 28 (46.7%) | 12 (42.9%) | 4 (14.3%) | 8 (28.6%) | 16(57.1) | 4 (14.3%) | 12 (42.9%) | |
| Moderate | 12 (20%) | 8 (66.7%) | 4 (14.3%) | 4 (33.3%) | 4 (33.3%) | 2 (16.7%) | 2 (16.7%) | |
| Strong | 16 (26.7%) | 4 (25%) | 0 (33.3%) | 4(25%) | 12 (75%) | 2 (12.5%) | 10 (62.5%) | |
| P63 | | | | | | | | |
| Negative | 12 (20%) | 0 (0%) | 0 (0%) | 0 (0%) | 12 (100%) | 4 (33.3%) | 8 (66.7%) | |
| Weak | 12 (20%) | 6 (50%) | 0 (0%) | 6 (50%) | 6 (50%) | 0 (0%) | 6 (50%) | <0.001§ |
| Moderate | 16 (26.7%) | 12 (75%) | 2 (12.5%) | 10 (62.5%) | 4 (25%) | 4 (25%) | 0 (0%) | |
| Strong | 20 (33.3%) | 18 (90%) | 10 (50%) | 8 (40%) | 2 (10%) | 2 (10%) | 0 (0%) | |

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD & median (range); •Kraskall Wallis H test; ‡ Chi-square test; § Chi-square test for trend; $p<0.05$ is significant

**2- Immunohistochemical expression of C-kit:** Regarding immunohistochemical expression of C-kit, cases were alienated into 2 groups: Negative (the negative and weak) C-kit expression group, and positive (the moderate and strong) C-kit expression group.

It was negative in 53.3% of cases and positive in 46.7% of cases. There was a significant association between the tumor grade and c-kit expression where it was positive in 4/24(16.7%) of low grade cases compared to 24/36(66.7%) of high grade cases with a statistically significant relationship ($p<0.001$). As regards the relationship between C-kit and stage, it tends to be less expressed in non-invasive cases compared to invasive UC with a statistically significant difference ($p<0.001$), as it showed positivity in only 2 cases out of 14 cases of non-invasive bladder carcinoma (14.7%), while it was positive in 56.5% of invasive cases, with 80% of T3 cases showing strong expression .C-kit expression was also correlated with patient' age ($p=0.015$) and sex ($p=0.011$) (Figure 2,Table 3).



(a) (b)

(c) (d)

***Figure2: Immunohistochemical expression of C-kit in bladder urothelial carcinoma*: (**a) Low grade urothelial carcinoma showing weak cytoplasmic immunoreactivity for C-kit. (b) Low grade urothelial carcinoma showing moderate cytoplasmic and membranous immunoreactivity for C-kit. (c) High grade urothelial carcinoma showing strong cytoplasmic and membranous immunoreactivity for C-kit. (d) High grade urothelial carcinoma showing strong cytoplasmic immunoreactivity for C-kit. (x400)

**Table 3:** Relation between clinicopathological features and immunohistochemical expression of C-kit in 60 patients with bladder urothelial carcinoma

| Characteristics | All (N=60) | C-kit | | | | | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | Negative (N=32) | Negative (N=18) | Weak (N=14) | Positive (N=28) | Moderate (N=12) | Strong (N=16) | |
| | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | |
| Age (years) | | | | | | | | |
| Mean ± SD | 62.1 ±7.46 | 63.2 ±6.78 | 65.3±6.25 | 60.4±6.63 | 60.8±8.11 | 58.2±5.70 | 62.8±9.22 | |
| Median (Range) | 61 (44-76) | 61 (53-76) | 64 (57-76) | 59 (53-75) | 61 (44-75) | 60 (47-63) | 61 (44-75) | 0.015• |
| ≤ 60 years | 28 (46.7%) | 14 (50%) | 4 (14.3%) | 10(35.7%) | 14 (50%) | 6 (21.4%) | 8 (28.6%) | |
| > 60 years | 32 (53.3%) | 18 (56.3%) | 14 (43.8%) | 4 (12.5%) | 14 (43.8%) | 6 (18.8%) | 8 (25%) | 0.048‡ |
| Sex | | | | | | | | 0.011‡ |
| Male | 48 (80%) | 26 (54.2%) | 14 (29.2%) | 12 (25%) | 22 (45.8%) | 6 (12.5%) | 16(33.3) | |
| Female | 12 (20%) | 6 (50%) | 4 (33.3%) | 2 (16.7%) | 6 (50%) | 6 (50%) | 0 (0%) | |
| Grade | | | | | | | | <0.001‡ |
| Low grade | 24 (40%) | 20 (83.3%) | 16 (66.7%) | 4 (16.7%) | 4 (16.7%) | 4 (16.7%) | 0 (0%) | |
| High grade | 36 (60%) | 12 (33.3%) | 2 (5.6%) | 10 (27.8%) | 24 (66.7%) | 8 (22.2%) | 16 (44.4 | |
| Stage | | | | | | | | <0.001§ |
| Non-invasive (Ta) | 14 (23.3%) | 12 (85.7%) | 10 (71.4%) | 2 (14.3%) | 2 (14.3%) | 2 (14.3%) | 0 (0%) | |
| Invasive | 46 (76.7%) | 20 (43.5%) | 8 (17.4%) | 12 (26.1%) | 26 (56.5%) | 10(21.7%) | 16 (34.8) | |
| T1 | 10 (16.7%) | 8 (80%) | 6 (60%) | 2 (20%) | 2 (20%) | 2 (20%) | 0 (0%) | |
| T2a | 2 (3.3%) | 2 (100%) | 0 (0%) | 2 (100%) | 0 (0%) | 0 (0%) | 0 (0%) | |
| T2b | 14 (23.3%) | 8 (57.1%) | 2 (14.3%) | 6 (42.9%) | 6 (42.9%) | 6 (42.9%) | 0 (0%) | |
| T3 | 20 (33.3%) | 2 (10%) | 0 (0%) | 2 (10%) | 18 (90%) | 2 (10%) | 16 (80%) | |
| Her2/Neu | | | | | | | | 0.001§ |
| Negative | 36(60%) | 24 (66.7%) | 12 (33.3%) | 12 (33.3%) | 12 (33.3%) | 8 (22.2%) | 4 (11.1%) | |
| 0 | 12 (20%) | 8 (66.7%) | 6 (50%) | 2 (16.7%) | 4 (33.3%) | 4 (33.3%) | 0 (0%) | |
| +1 | 24 (40%) | 16 (66.7%) | 6 (25%) | 10 (41.7%) | 8 (33.3%) | 4 (16.7%) | 4 (16.7%) | |
| Positive | 24 (40%) | 8 (33.3%) | 6 (25%) | 2 (27.8%) | 16 (66.7%) | 4 (16.7%) | 12 (50%) | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| +2 | 10 (16.7%) | 6 (60%) | 4 (40%) | 2 (20%) | 4 (40%) | 2 (20%) | 2 (20%) | |
| +3 | 14 (23.3%) | 2 (14.3%) | 2 (14.3%) | 0 (0%) | 12 (85.7%) | 2 (14.3%) | 10 (71.4) | |
| P63 | | | | | | | | 0.001§ |
| Negative | 12 (20%) | 2 (16.7%) | 0 (0%) | 2 (16.7%) | 10 (83.3%) | 0 (0%) | 10(83.3) | |
| Weak | 12 (20%) | 4 (33.3%) | 2 (16.7%) | 2 (16.7%) | 8 (66.7%) | 4 (33.3%) | 4 (33.3%) | |
| Moderate | 16 (26.7%) | 10 (62.5%) | 4 (25%) | 6 (37.5%) | 6 (37.5%) | 4 (25%) | 2 (12.5%) | |
| Strong | 20 (33.3%) | 16 (80%) | 12 (60%) | 4 (20%) | 4 (20%) | 4 (20%) | 0 (0%) | |

Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD & median (range);
•Kraskall Wallis H test; ‡ Chi-square test; § Chi-square test for trend; $p<0.05$ is significant.

**3- Immunohistochemical expression of P63:** P63 was expressed in 34/46 cases of invasive UC (73.9%) with variation in its expression as it was strongly expressed in 10/46 (21.7%), moderately expressed in 12/46 (26.1%), weakly expressed in 12/46 (26.1%) and was negative in 12/46 (26.1%) of cases. P63 was expressed in all non-invasive UC cases, as it was strongly expressed in 10/14 (71.4%), moderately expressed in 4/14 (28.6%) of cases.
P63 showed variation in its expression in high-grade UC, as it was strongly expressed in 6/36, moderately expressed in 10/36, weakly expressed in 8/36 and was not expressed in 12/36 (33.3%) of cases, while it was expressed in all low grade cases with strong expression in 14/24, moderate expression in 6/24 cases, weak expression in 4/24 cases. There was a statistically significant correlation between P63 expression and tumor grade ($p=0.001$) and tumor stage ($p<0.001$) (decreased expression with increasing grade and stage). There was a statistically significant association between P63 expression and age ($p=0.012$), but was not significant with sex ($p=0.105$) (Figure 3, Table 4).

**4- Correlation between Her2/Neu, C-kit and P63 immunohistochemical expressions:** Based on correlation analysis of markers expression among the studied cases, using Kendall's tau-b correleation coefficient, a significant positive correlation was detected between Her2/Neu and C-kit expression ( $\tau$ tau correleation coefficient = +0.35 ,$p=0.001$), while a significant strong inverse correlation was detected between P63 and both Her2/Neu ( $\tau$ tau correleation coefficient =-0.641 ,$p<0.001$), and C-kit ( $\tau$ tau correleation coefficient =-0.558 ,$p<0.001$) (Table 5).

**Table 4:** Relation between clinicopathological features and immunohistochemical expression of P63 in 60 patients with bladder urothelial carcinoma

| Characteristics | All (N=60) | P63 | | | | p-value |
|---|---|---|---|---|---|---|
| | | Negative (N=12) | Weak (N=12) | Moderate (N=16) | Strong (N=20) | |
| | No. (%) | No. (%) | No. (%) | No. (%) | No. (%) | |
| Age (years) | | | | | | 0.385• |
| Mean ± SD | 62.07±7.46 | 63.0±10.75 | 60.33±8.91 | 62.88 ±5.85 | 61.9±5.47 | 0.012‡ |
| Median (Range) | 61.0 (44-76) | 64.00 (44-75) | 60 (47-76) | 62.50 (53-75) | 59.50 (57-74) | |
| ≤ 60 years | 28 (46.7%) | 6 (21.4%) | 8 (28.6%) | 2 (7.1%) | 12 (42.9%) | |
| > 60 years | 32 (53.3%) | 6 (18.8%) | 4 (12.5%) | 14 (43.8%) | 8 (25%) | |
| Sex | | | | | | 0.105‡ |
| Male | 48 (80%) | 12 (25%) | 10 (20.8%) | 10 (20.8%) | 16 (33.3%) | |
| Female | 12 (20%) | 0 (0%) | 2 (16.7%) | 6 (50%) | 4 (33.3%) | |
| Grade | | | | | | 0.001‡ |
| Low grade | 24 (40%) | 0 (0%) | 4 (16.7%) | 6 (25%) | 14 (58.3%) | |
| High grade | 36 (60%) | 12 (33.3%) | 8 (22.2%) | 10 (27.8%) | 6 (16.7%) | |
| Stage | | | | | | <0.001§ |
| Non-invasive (Ta) | 14(23.3%) | 0 (0%) | 0 (0%) | 4(28.6%) | 10 (71.4%) | |
| Invasive | 46 (76.7%) | 12 (26.1%) | 12 (26.1%) | 12 (26.1%) | 10 (21.7%) | |
| T1 | 10 (16.7%) | 0 (0%) | 0 (0%) | 2 (20%) | 8 (80%) | |
| T2a | 2 (3.3%) | 0 (0%) | 0 (0%) | 2 (100%) | 0 (0%) | |
| T2b | 14 (23.3%) | 2 (14.3%) | 6 (42.9%) | 4 (28.6%) | 2 (14.3%) | |
| T3 | 20 (33.3%) | 10 (50%) | 6 (30%) | 4 (20%) | 0 (0%) | |
| Her2/Neu | | | | | | <0.001§ |
| Negative | 36 (60%) | 0 (0%) | 6 (16.7%) | 12 (33.3%) | 18(50%) | |
| 0 | 12 (20%) | 0 (0%) | 0 (0%) | 2 (16.7%) | 10 (83.3%) | |
| +1 | 24 (40%) | 0 (0%) | 6 (25%) | 10 (41.7%) | 8 (33.3%) | |
| Positive | 24 (40%) | 12 (50%) | 6 (25%) | 4 (16.7%) | 2 (8.3%) | |
| +2 | 10 (16.7%) | 4 (40%) | 0 (0%) | 4 (40%) | 2 (20%) | |
| +3 | 14 (23.3%) | 8 (57.1%) | 6 (42.9%) | 0 (0%) | 0 (0%) | |
| C-kit | | | | | | |
| Negative | 32(53.3%) | 2(6.3%) | 4 (12.5%) | 10 (31.3%) | 16 (50%) | |
| Negative | 18(30%) | 0(0%) | 2 (11.1%) | 4 (22.2%) | 12 (66.7%) | |
| Weak | 14(23.3%) | 2 (14.3%) | 2 (14.3%) | 6 (42.9%) | 4 (28.6%) | |
| Positive | 28(46.7%) | 10 (35.7%) | 8 (28.6%) | 6 (21.4%) | 4 (14.3%) | <0.001§ |
| Moderate | 12(20%) | 0 (0%) | 4 (33.3%) | 4 (33.3%) | 4 (33.3%) | |
| Strong | 16(26.7%) | 10 (62.5%) | 4 (25%) | 2 (12.5%) | 0 (0%) | |

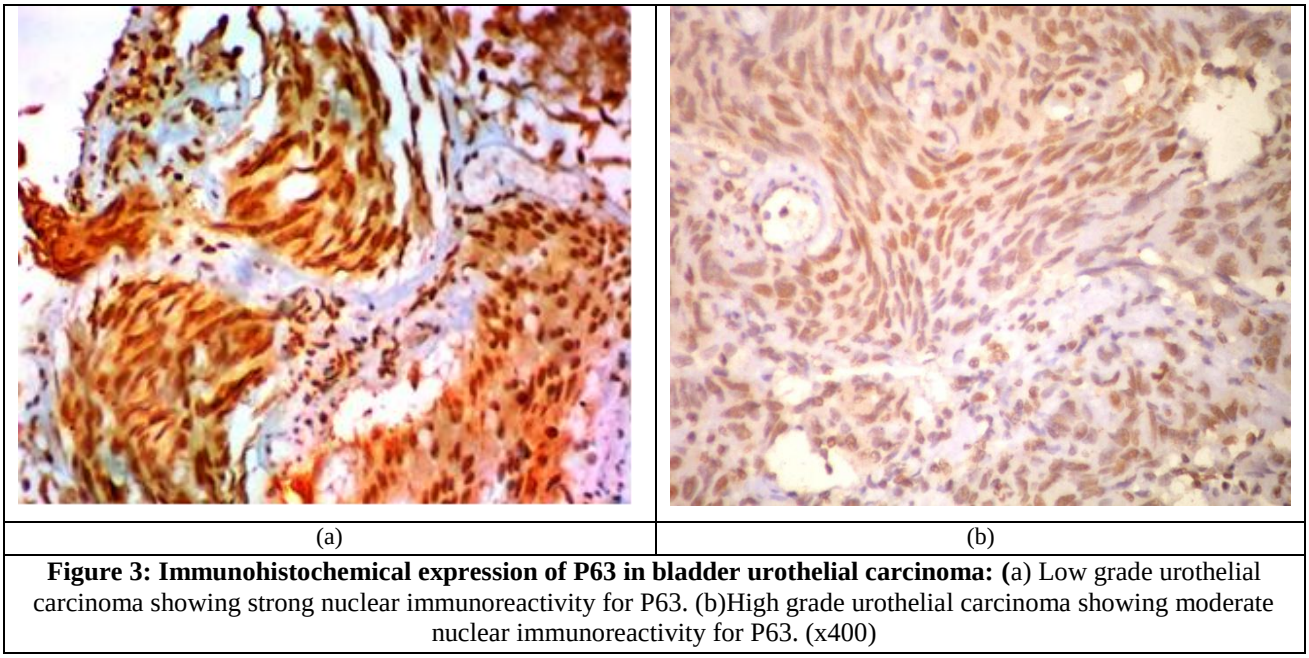
Categorical variables were expressed as number (percentage), continuous variables were expressed as mean ± SD & median (range);

• Kraskall Wallis H test; ‡ Chi-square test; § Chi-square test for trend; p<0.05 is significant.

**Table 5:** Correleation between Her2/Neu, C-kit and P63 immunohistochemical expression in 60 patients with bladder urothelial carcinoma

| | Her2/Neu | | C-kit | | P63 | |
|---|---|---|---|---|---|---|
| | τ | p-value | τ | p-value | τ | p-value |
| Her2/neu | --- | --- | +0.350 | 0.001 | -0.641 | <0.001 |
| C-kit | +0.350 | 0.001 | --- | --- | -0.558 | <0.001 |
| P63 | -0.641 | <0.001 | -0.558 | <0.001 | --- | --- |

τ tau correleation coefficient; p<0.05 is significant



(a) (b)

**Figure 3: Immunohistochemical expression of P63 in bladder urothelial carcinoma: (**a) Low grade urothelial carcinoma showing strong nuclear immunoreactivity for P63. (b)High grade urothelial carcinoma showing moderate nuclear immunoreactivity for P63. (x400)

## 5. Discussion

Cancer of the bladder is the fourth most common malignancy in males worldwide, and about 74,700 newly reported cases and 15,600 mortality due to urinary bladder carcinoma have been reported only in the USA during 2014 [32]. About 70 % of newly reported cases with UC of the bladder presented with low-grade carcinoma with no muscle invasion, of which 30% up to 70 % may recur, and about one-third of carcinoma may advance to high-grade or muscle-invasive bladder carcinoma. Cases diagnosed at the same grade or stage can have variant outcomes, which necessitate superior molecular markers for prognosis than existing clinical parameters for estimating the possibility of cancer advancement in these cases [33].

In our study, we address Her2/Neu, C-kit and P63 as important players in UC progression. The present research illustrates the expression of these markers in UC, followed by illustrating the association between their immunohistochemical expression and clinicopathological parameters.

Recently, immunohistochemical researches on UC have focus centered on the assessment of molecular markers responsible for the control of cellular cycles, to detect the predictive factors of these tumors. The prognostic significance of Her2/Neu in carcinoma of the bladder has not yet been fully recognized; though, in cancer breast, the achievement of trastuzumab therapy has stimulated attention in discovering the ability of applying this therapy on patients with UC [34].

There is wide range of Her 2/Neu immunohistochemical expression in UC , however, numerous researches that used immunohistochemistry in assessing the overexpression of Her2/Neu in these tumors, have shown a wide range of positivity in contrast to researches on the analysis of gene [34,35]. This may be explained by the fact that, the overexpression of Her2/Neu can develop through a mixture of two mechanisms. Firstly, is amplification of gene but this is uncommon in UC [36]. Secondly, is transcription up regulation that points to a growth factor nature of the Her2/Neu protein. Thereafter, greater amounts of transcription factor, even without amplification of gene leads to increase the expression of Her2/Neu protein [37,38].

In our research, Her 2/Neu expression was evaluated using immunohistochemistry (IHC), the method most frequently utilized for its assessment in breast carcinoma. There was a positive staining of Her2/Neu in 24/60 (40%) of studied carcinomas. This result is in concordance with previously related studies [34, 35, 39, 40], approving the competence of

immunohistochemistry in the evaluation of Her2/Neu status in UC.

Overexpression of Her2/Neu in breast, liver, ovarian, pancreatic and prostate malignant tumors, has been linked to poor outcomes [41].However, marked variation in the prognostic role of immunoexpression of Her2/Neu in UC was found in the results of previous related researches. Thus, although numerous researches [36, 42, 43], have detected a negative prognostic significance and an aggressive role of Her2/Neu expression in UC, others [41, 44], have not found any poor prognostic association. However, others [45, 46], have reported a better clinical outcome with Her2/Neu expression. In the current study, overexpression of Her2/Neu displayed a significant association with tumor grade ($p=0.023$) and the depth of tumor invasion ($p <0.001$), with more prevalent overexpression in deeply invasive and high grade tumors, pointing to the aggressive nature of the tumor cells. This is in concordance with the reports of numerous studies [41, 42, 43, 47, 48, 49], in which overexpression of Her 2/Neu signified the poor prognosis of the tumor. We reported no association between the expression of Her2/Neu and patient gender or age in our cohort ($P = 0.1$, 0.23 respectively), in agreement with El Gehani et al. [34]

These differences in the frequency and prognostic significance of Her2/Neu expression in bladder UC may be clarified by variant methods of assessment (gene amplification and protein overexpression), or using variant techniques (fluorescence in situ hybridization, and immunohistochemistry), variant clones of primary antibodies used and also different methods for scoring of IHC positivity [34,47].

Our analysis recommend that the expression of Her 2/Neu can present additional data for prognosis of bladder carcinoma cases, being more expressed in large percentage of patient in association with unfavorable outcome. Furthermore, bladder cancer cases must be checked for Her 2/Neu status for proper selection of patients who can get benefit from Her2/Neu targeted therapies following the radical surgery.

The proto-oncogene C-kit encodes a membrane receptor tyrosine kinase, which is linked to cell differentiation, proliferation, and control of apoptosis. C-kit expression in the urinary tract was described in chronic cystitis and nephrogenic metaplasia of the epithelial cells, probably because of mast cell migration and proliferation. However, reports on the expression of C-kit in bladder UC and the clinical implication of it are scarce [50].

In our study, there was a gradual increase in C-kit expression in parallel with the increase in the grade of tumor, where it showed positivity in 4/24(16.7%) of low grade cases compared to 24/36 (66.7%) of high grade cases with a significant difference ($p<0.001$). This fact was consistent with Aliza et al.[50], who found a significant difference between the tumor grade and C-kit positivity ($p= 0.001$). On the contrary, others [51] reported that overexpression of C-kit is not correlated with tumor grade. Furthermore, a significant association between C-kit expression in bladder carcinomas, and advanced tumor stage (depth of invasion) was noticed. The more invasive tumors (T2, T3) showed overexpression of C-kit than superficial tumors (Ta and T1), as it showed positivity in only 2 cases out of 14 cases of non-invasive UC (14.7%),while it was positive in 56.5% of invasive cases, with 80% of T3 cases showing strong expression with significant difference between stages ($p<0.001$).This result was consistent with Aliza et al.[50]. On the other hand, Pan et al.,[52] reported no significant association between overexpression of C-kit and tumor stage.

These findings support the function of C-kit in the carcinogenesis of UC regarding behavior, and aggressiveness, and thus C-kit could be considered as a poor prognostic parameter in urinary bladder carcinoma.

Therapy with KIT tyrosine kinase inhibitors (TKIs) will be successful only for neoplasms in which growth is stimulated nearly by KIT action. Consequently, in choosing tumor targets that will respond to therapy with TKIs, the best responsive targets will be cancers for which there are data pointing that the cells are partially or completely based on KIT stimulation for or survival and proliferation[51].

P63 has emerged as an important player in embryonic development, epithelial stem cell maintenance and differentiation. In cancer biology, it has been implicated in all steps of tumorgenesis and progression of malignancy [53]. The gene encoding P63 protein is a one of the p53 family and have two diverse promoters that create two types of P63 proteins; the trans activating TAP63 and the NH2-terminal truncated ΔNP63[54]. P63 regulates many genes that function in DNA repair, ΔNP63 fixes to the promoters of the BRCA2, RAD51, and MRE11 genes that are implicated in homologous recombination, which is one of the most essential pathways for double-strand breaks repair. Furthermore, P63 reacts with ATM, a key kinase that is implicated in the detection of the breaks of DNA double-strand [53].

It has been shown that reduced the expression of P63 is linked to progression and advanced stages of cancer breast [55, 65] and melanoma [57]. Elnashar et al. [31] reported P63 expression in all normal and hyperplastic urothelium in the areas adjacent to the tumor in their studied cases. An earlier study at 2001 revealed that P63 is essential for the maintenance and proliferation of epithelial progenitor cells that generate to the mature stratified squamous epithelial cells rather than for the maturation process itself [58]**.** On the other hand, a study group reported that P63 expression was correlated with the degree of differentiation in the superficial lesion and with the number of cell layers which covered the tumor papillae in muscle-invasive UC [59]. Many reports which tried to evaluate P63 role in tumergenesis recommend that it is used in cell adhesion and migration, thus also in processes related to these cell abilities as metastasis. Studies that has been performed on squamous cell carcinoma lines have suggested that P63 disruption causes upregulation of genes having a higher potential to invasion and metastasis [60]**,** while a more recent study reported that TAP63 inhibits metastasis by controlling micro RNA complex processing [61].

In our study, P63 was expressed in 34/46 cases of invasive UC (73.9%) with variation in its expression as strongly, moderately or weakly expressed and was negative in 12/46 (26.1%) of cases, while it was expressed in all of non-invasive UC cases with a statistically significant relationship (p value <0.001). These results are in agreement with other authors [62] who found P63 expression in all cases of non-invasive UC with strong immune reaction in low grade papillary superficial carcinoma (93%) than in high grade UC (68%) that showed a significant reduction in P63 expression. On the other hand, Koyuncuer in his study found no statistically significant differnce between invasive and non-invasive UC regarding P63 expression [63].

P63 positive expression was detected in all cases of low grade UC and in (66.7%) of high-grade UC with a significant relationship between P63 staining and tumor grading (p =0.001) (decreased expression with increasing grade), this was in concordance with two studies which concluded that P63 expression diminished in high grade invasive UC [64, 65].

A study was conducted in 2012 concluded that it is impossible to detect fatal tumors that invade the muscle prospectively; however, collecting data supposes that molecular programming that characterize the process of epithelial-to mesenchymal transition (EMT) is implicated. Invasive tumors are characterized by down-regulation of P63 and E-cadherin, two epithelial markers that are regularly presented in normal urothelium and in tumors with no muscle invasion. These alterations are associated with up-regulation of mesenchymal markers such as Zeb-1, Zeb-2, MMP9 and vimentin, responsible for increased invasion and migration [66].

Our results are similar to a very recent study in 2016 [31] who reported that P63 was expressed in 28/38 cases (73.7%) of invasive UC and in all cases of non-invasive UC, and also in 16/17 (94%) of low-grade and in 24/33 (72.7%) of high-grade UC, with a significant relation between P63 expression and both invasion (p=0.0001) and the tumor grade (p= 0.034), thus, P63 can be considered as a poor prognostic marker in UC.

## 6. Conclusion

Our findings support the role of Her2/Neu, C-kit overexpression and loss of P63 in the carcinogenesis of urothelial carcinoma regarding behavior, and aggressiveness, and thus could be considered as a poor prognostic parameter. A significant correlation was found between immunohistochemical expression of Her2/Neu and the WHO grade of urothelial carcinoma, thus Her2/Neu as a marker can be used to aid in assessing high grade urothelial tumors in controversial cases in which the decision between low and high grade urothelial tumors is crucial. Furthermore, cases with urothelial carcinoma must be examined for Her-2/Neu and C-kit status for selection of proper candidates who may benefit from adjuvant Her 2/Neu and C-kit targeted therapies.

## References

[1] Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, Kassouf W, Kiemeney LA, La Vecchia C, Shariat S, Lotan Y. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 2013; 63:234-241.
[2] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010; 127(12):2893-917.
[3] Huang W, Williamson SR, Rao Q, Lopez-Beltran A, Monitroni R, Eble JN, Grignon DJ, Idrees MT, Emerson RE, Zhou XJ, Zhang S, Baldridge LA, Hahn NM, Wang M, Koch MO, Cheng L. Novel markers of squamous differentiation in the urinary bladder. Hum Pathol. 2013; 44:1989-1997.
[4] Gouda I, Mokhtar N, Bilal D, El-Bolkainy T, El-Bolkainy NM. Bilharziasis and bladder cancer: a time trend analysis of 9843 patients. J Egypt Natl Canc Inst. 2007; 19:158-162.
[5] Khaled H. Schistosomiasis and cancer in Egypt: review. Medical Oncology, National Cancer Institute, Cairo University, Cairo 11796, Egypt. J Advanced Res. 2013; 4:461-466.
[6] Youssef RF, Lotan Y. Predictors of outcome of non-muscle-invasive and muscle-invasive bladder cancer. Sci World J. 2011; 11:369–381.
[7] Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. Nat Rev Cancer. 2015; 15(1):25–41.
[8] Nedjadi T, Al-Maghrabi J, Assidi M, Dallol A, Al-Kattabi H, Chaudhary A, Al-Sayyad A, Al-Ammari A, Abuzenadah A, Buhmeida A , Al-Qahtani M. Prognostic value of HER2 status in bladder transitional cell carcinoma revealed by both IHC and BDISH techniques BMC Cancer. (2016) 16:653.
[9] Joensuu H, Kellokumpu-Lehtinen PL, Bono P, Alanko T, Kataja V, Asola R, Utriainen T, Kokko R, Hemminki A, Tarkkanen M, Turpeenniemi-Hujanen T, Jyrkkio S, Flander M, Helle L, Ingalsuo S, Johansson K, Jaaskelainen AS, Pajunen M, Rauhala M, Kaleva-Kerola J, Salminen T, Leinonen M, Elomaa I, Isola J Adjuvant docetaxel or vinorelbine with or without trastu-zumab for breast cancer. N Engl J Med .2006 ;354(8):809–820.
[10] Yarden Y. Biology of HER2 and its importance in breast cancer. Oncology. 2001; 61(2):1.
[11] Yoshizawa A, Sumiyoshi S, Sonobe M, Kobayashi M, Uehara T, Fujimoto M, Tsuruyama T, Date H, Haga H. HER2 status in lung adenocarcinoma: a comparison of immunohistochemistry, fluorescence in situ hybridization (FISH), dual-ISH, and gene mutations. Lung Cancer. 2014; 85(3):373-378.
[12] Bahmani B, Guerrero Y, Bacon D, Kundra V, Vullev VI, Anvari B Functionalized polymeric nano particles loaded with indo-cyanine green as theranostic materials for targeted molecular near infrared fluorescence imaging and photothermal destruction of ovarian cancer cells. Lasers Surg Med. 2014; 46(7):582-592.
[13] Drecoll E, Nitsche U, Bauer K, Berezowska S, Slotta-Huspenina J, Rosenberg R, Langer R. Expression analysis of heat shock protein 90 (HSP90) and Her2 in

colon carcinoma. Int J Colorectal Dis .2014;29(6):663–671.
[14] Ettl T, Stiegler C, Zeitler K, Agaimy A, Zenk J, Reichert TE, Gosau M, Kuhnel T, Brockhoff G, Schwarz S. EGFR, HER2, survivin, and loss of pSTAT3 characterize high-grade malignancy in salivary gland cancer with impact on prognosis. Hum Pathol. 2012; 43(6):921–931.
[15] Kruger S, Weitsch G, Buttner H, Matthiensen A, Böhmer T, Marquardt T, Sayk F, Feller AC, Böhle A.. Overexpression of c-erbB-2 oncoprotein in muscle-invasive bladder carcinoma: relationship with gene amplification, clinicopathological parameters and prognostic outcome. Int J Oncol. 2002; 21(5):981-987.
[16] Kolla SB, Seth A, Singh MK, Gupta NP, Hemal AK, Dogra PN, Kumar R. Prognostic significance of Her2/neu overexpression in patients with muscle invasive urinary bladder cancer treated with radical cystectomy. Int Urol Nephrol. 2008; 40(2):321-327.
[17] Chow NH, Chan SH, Tzai T, Ho CL, Liu HS. Expression profiles of ErbB family receptors and prognosis in primary transitional cell carcinoma of the urinary bladder. Clin Cancer Res. 2001; 7(7):1957-1962.
[18] Liedberg F, Anderson H, Chebil G, Gudjonsson S, Höglund M, Lindgren ,Lundberg L, Lövgren K.Tissue microarray based analysis of prognostic markers in invasive bladder cancer:much effort to no avail? Urol Oncol. 2008; 26:17-24.
[19] Kassouf W, Black PC, Tuziak T, Bondaruk J, Lee S, Brown G, Adam L, Wei C, Baggerly K, Bar-Eli M, McConkey D, Czerniak B, Dinney C. Distinctive expression pattern of ErbB family receptors signifies an aggressive variant of bladder cancer. J Urol. 2008; 179(1) :353-358.
[20] Tian Q, Frierson HF Jr, Krystal GW, Moskaluk CA. Activating c-KIT gene. Mutations in human germ cell tumors. Am. J. Pathol. 1999; 154(6): 1643-1647,
[21] Tsuura Y, Hiraki H, Watanabe K, Igarashi S, Shimamura K, Fukuda T, Suzuki T, Seito T : Pref-erential localization of c-KIT product in tissue mast cells,basal cells of skin, epithelial cells of breast, small cell lung carcinoma and seminoma/dysgerminoma in human: Immunohistochemical study on formalin-fixed, paraffin-embedded tissues. Virchows. Arch. 1994; 424: 135-141.
[22] Shams T and Metawea M. Overexpression of C-KIT in Schistosomal Urinary Bladder Carcinoma Med. J. Cairo Univ.2011;79(1):449-455.
[23] Hodges KB, Lopez-Beltran A, Emerson RE, Montironi R, Cheng L. Clinical utility of immunohistochemistry in the diagnoses of urinary bladder neoplasia. Appl Immunohistochem Mol Morphol. 2010;18:401-410.
[24] Pignon J C, Grisanzio C, Geng Y, Song J,Shivdasani RA, Signoretti S. P63-expressing cells are the stem cells of developing prostate, bladder, and colorectal epithelia. Proc Natl Acad Sci U S A. 2013; 110 (20): 8105-8110.
[25] Gressner O, Schilling T, Lorenz K, Schulze-Schleithoff E, Koch A, Schulze-Bergkamen H, Lena AM, Candi E, Terrinoni A, Catani MV, Oren M, Melino G, Krammer PH, Stremmel W, Muller M. TAp63α induces apoptosis by activating signaling via death receptors and mitochondria. EMBO J. 2005; 24: 2458-2471.
[26] Hibi K., Trink, B., Patturajan M, Westra WH, Caballero OL, Hill DE, Ratovitski EA, Jen J, Sidransky D.AIS are an oncogene amplified in squamous cell carcinoma. Proc Natl Acad Sci. 2000; 97: 5462-5467.
[27] Lopez-Beltran A, Sauter G, Gasser T, Hartmann A, Schmitz-Drager BJ, Helpap B, Ayala AG, Tamboli P, Knowles MA, Sidransky D, Cordon-Cardo C, Jones PA, Cairns P, Simon R, Amin MB, Tyczynski JE. Infiltrating urothelial carcinoma: In World Health Organization classification of tumors. Pathology and Genetics of tumors of the urinary system and male genital organs. Lyon, IARCC Press. 2004; PP: 91-94.
[28] Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A. AJCC cancer staging manual (7thed). New York, NY: Springer 2010.
[29] Shawkya A, El hafeza A,Elosailyb GM, Al-Matubsi HY, Farahat A. Her-2/Neu Overexpression in Invasive Bladder Carcinoma Among a Cohort of Egyptian Patients World J Nephrol Urol .2013;2(2):70-75.
[30] Tealeb AM. C-Kit and Bcl-2 in Bladder Carcinoma: An Immunohistochemical Study. AAMJ, 2013; 11(1).
[31] Elnashar AT, El-Naby NE, Nageeb OM. Abd El Wahab AG. Immunohistochemical Expression of P63 in Urothelial Carcinoma. Cancer Biology. 2016; 6(3).
[32] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015;65(1):5–29.
[33] Cho KS, Seo HK, Joung JY, Park WS, Ro JY, Han KS, Chung J, Lee KH. Lymphovascular invasion in transurethral resection specimens as predictor of progression and metastasis in patients with newly diagnosed T1 bladder urothelial cancer. J Urol.2009; 182(6):2625–2630.
[34] El Gehani K, Al-Kikhia L, Emaetig F, Syrjanen K, Al-Fituri O, Elzagheid A. Over-expression of HER-2 is associated with the stage in carcinomas of the urinary bladder. Libyan J Med. 2012;7:14964:1-6 .
[35] Naik DS, Sharma S, Ray A, Hedau S. Epidermal growth factor receptor expression in urinary bladder cancer. Indian J Urol. 2011;27(2):208-214.
[36] Kruger S, Weitsch G, Buttner H, Matthiensen A, Bohmer T, Marquardt T, Sayk F, Feller AC, Böhle A. HER2 overexpression in muscle-invasive urothelial carcinoma of the bladder: prognostic implications. Int J Cancer. 2002;102(5):514-518.
[37] Ohta JI, Miyoshi Y, Uemura H, Fujinami K, Mikata K,Hosaka M, Tokita Y. Fluorescence in situ hybrid-ization evaluation of c-erbB-2 gene amplification and chromosomal anomalies in bladder cancer. Clin Cancer Res. 2001;7(8):2463-2467.
[38] Latif Z, Watters AD, Dunn I, Grigor K, Underwood MA, Bartlett JM. HER2/neu gene amplification and protein overexpression in G3 pT2 transitional cell carcinoma of the bladder: a role for anti-HER2 therapy? Eur J Cancer.2004; 40(1):56-63.
[39] Gandour-Edwards R, Lara PN, Jr., Folkins AK, LaSalle JM, Beckett L, Li Y, Meyers FJ. Does HER2/neu expression provide prognostic information in pa-tients with advanced urothelial carcinoma? Cancer. 2002;95(5):1009-1015.

[40] Caner V, Turk NS, Duzcan F, Tufan NL, Kelten EC, Zencir S, Dodurga Y, et al. No strong association between HER-2/neu protein overexpression and gene amplification in high-grade invasive urothelial carcinomas. Pathol Oncol Res. 2008;14(3):261-266.
[41] Alexa A, Baderca F, Zahoi DE, Lighezan R, Izvernariu D, Raica M. Clinical significance of Her2/neu overexpression in urothelial carcinomas. Rom J Morphol Embryol. 2010;51(2):277-282.
[42] Skagias L, Politi E, Karameris A, Sambaziotis D, Archondakis A, Vasou O, Ntinis A, et al. Prognostic impact of HER2/neu protein in urothelial bladder cancer. Survival analysis of 80 cases and an overview of almost 20 years' research. J BUON. 2009;14(3):457-462.
[43] Enache M, Simionescu CE, Stepan A. EGFR and Her2/neu immunoexpression in papillary urothelial bladder carcinomas. Rom J Morphol Embryol. 2013;54(1):137-141.
[44] Jimenez RE, Hussain M, Bianco FJ Jr , Vaishampayan U, Tabazcka P, Sakr WA, Pontes JE. Her-2/neu overexpression in muscle-invasive urothelial carcinomaof the bladder: prognostic significance and comparative analysis in primary and metastatic tumors. Clin Cancer Res. 2001;7(8):2440-2447.
[45] Korkolopoulou P, Christodoulou P, Kapralos P, Exarchakos M, Bisbiroula A, Hadjiyannakis M, Georgountzos C. The role of p53, MDM2 and c-erb B-2 oncop-roteins, epidermal growth factor receptor and proliferation markers in the prognosis of urinary bladder cancer. Pathol Res Pract. 1997;193(11-12):767-775.
[46] Vollmer RT, Humphrey PA, Swanson PE, Wick MR, Hudson ML. Invasion of the bladder by transitional cell carcinoma: its relation to histologic grade and expression of p53, MIB-1, c-erb B-2, epidermal growth factor receptor, and bcl-2. Cancer. 1998;82(4):715-723.
[47] Atis G, Arisan S, Dalkilinc A. Determination of Her-2/Neu gene amplification and protein overexpression in bladder transitional cell carcinoma. Adv Mol Biol. 2007;1:89-94.
[48] Jalali Nadoushan MR, Taheri T, Jouian N, Zaeri F. Overexpression of HER-2/neu oncogene and transitional cell carcinoma of bladder. Urol J. 2007;4(3):151-154.
[49] Kolla SB, Seth A, Singh MK, Gupta NP, Hemal AK, Dogra PN, Kumar R. Prognostic significance of Her2/neu overexpression in patients with muscle invasive urinary bladder cancer treated with radical cystectomy. Int Urol Nephrol. 2008;40(2):321-327.
[50] Aliza Z, Gad N, Yoram D, Alon H, Tuvia H, Gideon SY, Edward Y, Lea RW .Expression of C-KIT receptor in transitional cell carcinoma of urinary bladder and its correlation with clinicopathological variables Conexunimedicale .2009; 4(16).
[51] Shams T and Metawea M. Overexpression of C-KIT in Schistosomal Urinary Bladder Carcinoma Med. J. Cairo Univ.2011; 79,(1): 449-455.
[52] Pan CX , Yang XJ, Lopez-Beltran A, MacLennan GT, Eble JN, Koch MO, Jones TD, Lin H, Nigro K, Papavero V, Tretiakova M, Cheng L.C-KIT expression in small cell carcinoma of the urinary bladder: Prognostic and therapeutic implications. Mod. Pathol. 2005; 18(3):320-323.
[53] Melino G. P63 is a suppressor of tumergenesis and metastasis interacting with mutant P53. Cell Death Differe. 2011; 18 (9): 1487-1499.
[54] Pignon J C, Grisanzio C, Geng Y, Song J,Shivdasani RA, Signoretti S. P63-expressing cells are the stem cells of developing prostate, bladder, and colorectal epithelia. Proc Natl Acad Sci U S A. 2013; 110 (20): 8105-8110.
[55] Stefanou D, Batistatu A, Nonni A, Arkoumani E, Agnantis NJ. P63 expression in benign and malignant breast lesions.Histol Histopathol. 2004; 19(2): 465-471.
[56] Wang X, Mori I, Tang W, Nakamura M, Nakamura Y, Sato M, Sakurai T, Kakudo K. P63 expression in normal, hyperplastic and malignant breast tissues. Breast Cancer 2002; 9(3): 216: 219
[57] Matin RN, Chikh A, Chong SL, Mesher D, Graf M, Sanza' P, Senatore V, Scatolini M, Moretti F, Leigh IM, Proby CM, Costanzo A, Chiorino G, Cerio R, Harwood CA, Bergamaschi D. p63 is an alternative p53 repressor in melanoma that confers chemoresistance and a poor prognosis. J Exp Med. 2013; 11:210(3):581-603
[58] Pellegrini G, Dellambra E, Golisano O, Martinelli E, Fantozzi I, Bondanza S, Ponzin D, McKeon F and DeLuca M. P63 identifies keratinocytes stem cells. Pro Natl AcadSci USA 2001; 98: 3156-3161.
[59] Stepan A, Margaritescu CL, Simionescu C, Ciurea R. E-cadherin and P63 immunoexpression in dysplastic lesions and urothelial carcinoma of the bladder. Romanian J of Morphology and Embryology .2009; 50 (3): 461-465.
[60] Barbieri CE, Tang LJ, Brown KA, and Pietenpol JA. Loss of p63 leads to increased cell migration and upregulation of genes involved in invasion and metastasis. Cancer Res 2006; (66): 7589-7597.
[61] Su X, Chakravarti D, Cho MS, Liu L, Gi YJ, Lin YL, Leung ML, El-Naggar A, Creighton CJ, Suraokar MB, Wistuba I. Flores ER. TAp63 suppresses metastasis through coordinate regulation of Dicer and mi RNAs. Nature 2010; 467: 986-990.
[62] Raheem SA, Saied AN, Al Shaer R, Mustafa O, Ali AH. The role of CK20, P53 and P63 in differentiation of some urothelial lesions of urinary bladder, immunohistochemical study. Open Journal Of Pathology 2014; 4: 181-193.
[63] Koyuncuer A. Immunohistochemical expression of P63 and P53 in urinary bladder carcinoma. Indian J Pathology & Microbiology. 2013; 56 (1): 10-15.
[64] Urist MJ, Di Como CJ, Lu ML, Charytonowicz E, Verbel D, Crum CP, Ince TA, Mckeon FD, Cordon-Cardo C. Loss of P63 expression is associated with tumor progression in bladder cancer. Am J Patholo. 2002; 16 (4): 1199-1206.
[65] Koga F, Kawakami S, Fuji Y, Sato K, Ohtsuka Y, Iwai A, Ando N, Takizawa T, Kaqeyama Y, Kihara K. Impaired p63 expression associated with poor prognosis and Uroplakin III expression in invasive urothelial carcinoma of the bladder. Clin Cancer Res 2003; 9(15): 5501-5507.
[66] Choi W, Shah JB, Tran M, Svatek R, Marquis L, Lee L, Yu D, Adam L, Wen S, Shen Y, Dinney C, McConkey DJ, Siefker-Radtke A. P63 expression defines lethal subsets of muscle-invasive bladder cancer. Plosone 2012; 7 (1): e30206.