

Her2/ Neu and Ki -67 Immunohistochemical Expression in Transitional Cell Carcinoma of the Urinary Bladder (A Clinicopathological Study)

Noora Ali Jawad¹, Dr. Hussam Hasson Ali², Mohammed Subhi Kamal³

¹M.B.Ch.B.,

²Professor M.B.Ch.B., M.Sc, F.I.C.M.S (Path.),

³M.B.Ch.B., F.I.C.M.S (Path.)

Summary

Background: Urinary bladder carcinoma is one of the most common cancer worldwide, it is the fourth most common cancer in men and the eighth in women in the world. In Iraq bladder carcinoma is the second most common cancer in males and the tenth in females according to Iraqi Cancer Registry. About 95% of bladder tumors are of epithelial origin, and TCC accounts for 90% of all bladder cancers. Few studies had been done about the role of (Her2/Neu and Ki-67) in urothelial carcinoma of the bladder. The present study has used the 2004 WHO grading system of urothelial carcinoma and the AJCC/UICC T staging system of the urothelial carcinoma of the bladder.

Aim Of The Study: To study the immunohistochemical expression of proliferation marker kinase inhibitor 67 (Ki-67) and human epidermal growth factor receptor-2 (Her-2/Neu) in non-tumorous uroepithelium as compared with different stages and grades of urothelial carcinoma, with special emphasis on low grade and high grade lesions to reveal their help as an ancillary technique in the diagnosis.

Samples, Materials And Methods : In this retrospective study covered the period from June 2014 to October 2015, formalin- fixed paraffin-embedded tissue blocks were collected from the archived materials from the specialized surgical hospital. The paraffin blocks represented (45) cases of urothelial carcinoma of the bladder removed surgically by TURBT. The histopathological diagnosis had been revised and all cases were stained by immunohistochemical technique with Her-2/Neu and Ki-67 tumor markers. Values were considered statistically significant when $P < 0.05$.

Results: Immunohistochemical expression of Her-2/Neu and Ki-67 revealed that there was no significant correlation between the expression of these markers with the age and gender (P value > 0.05). There was significant correlation between the expression of Her-2/Neu (P value 0.018) and Ki-67 (P value 0.013) with the 2004 WHO grading system of urothelial carcinoma. Regarding immunohistochemical expression of Her-2/Neu and Ki-67 with the AJCC/UICC T staging system of the urothelial carcinoma of the bladder revealed that there was no significant correlation between the expression of both (Her-2/Neu and Ki-67) and the stage (P value > 0.05).

Conclusions: A significant correlation was found between immunohistochemical expression of Her2/neu and the WHO 2004 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. A significant correlation was found between ki-67 immunohistochemical expression and the WHO 2004 grade of urothelial carcinoma, hence, ki-67 can be used in the assessment of controversial high grade urothelial tumors.

I. Introduction

Urinary bladder carcinoma is one of the most common cancer worldwide, it is the fourth most common cancer in men and the eighth in women in the world. ⁽¹⁾ In Iraq bladder carcinoma is the second most common cancer in males and the tenth in females according to Iraqi Cancer Registry. ⁽²⁾ About 95% of bladder tumors are of epithelial origin ⁽³⁾, and TCC accounts for 90% of all bladder cancers. ^(4,5) Nearly 80% of patients fall between 50 and 80 years of age. ⁽⁶⁾ With a male to female ratio of 3:1. ⁽⁷⁾ Known prognostic factors for bladder cancer survival include tumor stage and tumor grade. Histological tumor grade is considered an important prognostic factor in particular for the non-muscle-invasive urothelial bladder neoplasms (NMIBC), a category that includes tumors either with only lamina propria invasion or without invasion. It is difficult to predict accurate prognosis with any single factor. However, accurate estimation of the biological behavior of these tumors is important to select the appropriate treatment. Therefore, more reliable prognostic factors are urgently needed, the prime interest being currently focused on protein and genetic markers. ^(8,9)

Aim Of The Study

To study the immunohistochemical expression of proliferation marker kinase inhibitor 67 (Ki-67) and human epidermal growth factor receptor-2 (Her-2/Neu) in non-tumorous uroepithelium as compared with different stages and grades of urothelial carcinoma, with special emphasis on low grade and high grade lesions to reveal their help as an ancillary technique in the diagnosis.

Review Of Literature

1.1 Incidence And Epidemiology Of Urinary Bladder Transitional Cell Carcinoma

Urothelial carcinomas are the fourth most common tumors after prostate (or breast), lung and colorectal cancer. ^(10, 11) They can be located in the lower urinary tract (bladder and urethra) or upper urinary tract (pyelocaliceal cavities and ureter). Bladder tumours account for 90-95% of urothelial carcinomas and are the most common malignancy of the urinary tract. ^(11, 12)

Bladder cancer is the fourth most commonly diagnosed malignancy in men in the United States. It is estimated that 74,690 new cases of bladder cancer are expected to occur in the United States in 2014. ⁽¹³⁾ Since the 1950s, the incidence of bladder cancer has risen by approximately 50%. It is to be anticipated that, with the aging of the U.S.

Bladder cancer is diagnosed almost twice as often in whites as in blacks of either sex. The incidence of bladder cancer among other ethnic and racial groups in the United States falls between that of blacks and whites. The incidence of bladder cancer increases with age. ⁽¹⁴⁾

In Iraq, the incidence of most types of cancer (including bladder cancer) has increased sharply in the last few years due to exposure to wars pollution . Most of patients were from the Middle Euphrates area and south of Iraq. Both regions were potentially exposed to environmental pollution during the last two decades of wars. The patients ages ranged between 35 and 85 years, with a median age of 69.3 years. ⁽¹⁵⁾ Cancer of the urinary bladder is the top cancer in males in Basrah during the years 2005- 2008. It accounts for 13.7% of all cancer cases among males and it is the fifth most common malignancy (4.1%) in females. ⁽¹⁶⁾

1.2 WHO (2004)/ISUP classification system.

The 2004 WHO classification of the papillary urothelial lesions grading differentiates between papillary urothelial neoplasm of low malignant potential (PUNLMP) and low-grade and high-grade urothelial carcinomas. ⁽¹⁷⁻¹⁸⁾

Papillary Urothelial Carcinoma, Low-Grade: Low-grade papillary urothelial carcinomas are characterized by an overall orderly appearance but with easily recognizable variation of architectural and or cytologic features seen at scanning magnification. Variation of polarity and nuclear size, shape, and chromatin texture comprise the minimal but definitive cytologic atypia. Mitotic figures are infrequent ⁽¹⁹⁾

Papillary-Urothelial Carcinoma, High-Grade: Predominantly or totally disorderly appearance at low magnification with both architectural and cytologic abnormalities. Architecturally cells appear irregularly clustered and the epithelium is disorganized. Cytologically, there is a spectrum of pleomorphism .The nuclear chromatin tends to be clumped and nucleoli may be prominent. Mitotic figures, including atypical forms, are frequently seen at all levels of the spectrum of cytologic atypia within high-grade papillary urothelial carcinomas. ⁽¹⁹⁾

Table.1.1 World Health Organization (WHO) Grading of Urinary Tumors in 2004 ⁽²⁰⁾

WHO 2004
• Urothelial papilloma
• PUNLMP
• Low-grade papillary urothelial carcinoma
• High-grade papillary urothelial carcinoma
PUNLMP = papillary urothelial neoplasms of low malignant

1.3 Human epidermal growth factor receptor 2(HER2/neu)

ERBB2, a known proto-oncogene, is located at the long arm of human chromosome 17 (17q12). HER2 is named because it has a similar structure to human epidermal growth factor receptor, or HER1. *Neu* is so named because it was derived from a rodent glioblastoma cell line, a type of neural tumor. *ErbB-2* was named for its similarity to *ErbB* (avian erythroblastosis oncogene B), the oncogene later found to code for EGFR. Gene cloning showed that HER2, Neu, and ErbB-2 are all encoded by the same orthologs. ⁽²¹⁾

Immunohistochemical Studies Of Her2/Neu Marker In Urothelial Carcinoma Of The Bladder

Lately, many studies are concentrated on the evaluation of certain markers involved in the regulation of the cell cycle, among them being the human epidermal receptors (HER) ⁽²²⁾, which can represent a promising therapeutical target. ElMoneim HMA *et al.* (2011) reports Her2/neu overexpression statistically correlated with high-grade tumors but did not find any correlation with the tumor stage. ⁽²³⁾

1.4 Proliferation Marker Kinase Inhibitor (Ki- 67)

Ki67 is a non-histone nuclear protein with a short life. ⁽²⁴⁾ That is encoded by the MKI67 gene in humans and is a cellular marker for proliferation. It is strictly associated with cell proliferation, which is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). ⁽²⁴⁾

Possible Mechanisms Of Ki-67 In Urothelial Carcinoma

Although tumor grade and stage are considered signs of aggressive behavior for bladder cancer, several reports in the literature describe a correlation between Ki67 labeling index with well-known prognostic factors, such as grade and stage. ⁽²⁵⁾

The literature data indicates that Ki67 proliferation index has increased in high-grade carcinoma with or without invasion. ⁽²⁶⁾ Studies carried until now have established that it is an independent predictor for recurrence, progression and response to treatment in invasive urothelial carcinoma. ⁽²⁶⁾

Samples, Materials And Methods

2.1: Sample:

In this retrospective study covered the period from June 2014 to October 2015 , formalin- fixed paraffin-embedded tissue blocks were collected from the archived materials from the specialized surgical hospital in Baghdad /Iraq.

The paraffin blocks represented (45) cases of urothelial carcinoma of the bladder removed surgically by TURBT.

Three sections of 5 µm thickness were taken, the first was stained by hematoxylin and eosin (H&E) for histopathological revision; the other two sections were put on positively charged slides and stained immunohistochemically for her2/neu and Ki-67. The H&E stained sections were reassessed for histological typing, grading and staging of the tumors.

2.2. Materials:

2.2.1. Primary Antibodies (Her2/Neu And Ki-67) Tumor Markers:

- 1- Polyclonal Rabbit Anti-human c-erbB-2 oncoprotein is intended for laboratory use to identify qualitatively by light microscopy her2/neu positive cells in normal and neoplastic tissue using immunohistochemical (IHC) test methods .
- 2- Monoclonal Mouse Anti-Human Ki-67 antigen clone MIB-1, is intended for use in using immunohistochemistry (table 2.1).

2.2.2. Secondary Detection System:

[BioGenex Kit Fremont, CA94538USA].

- super Enhancer.
- ss LABEL (polymer-HRP).

Dako

- Liquid DAB+substrate.
- Chromogen system.

2.3 Assessment of Immunohistochemical Staining:

1.Her-2/neu:

Immunostained sections were evaluated under light microscope. The brown membranous staining intensity and pattern were considered for scoring according to Ventana pathway Her-2/neu protocol in the following scheme: 0, no staining or membrane staining observed in less than 10% of the tumor cells; 1+, partial faint membrane staining in more than 10% of the tumor cells; 2+, circumferential weak to moderate staining observed in more than 10% of the tumor cells; 3+, circumferential strong membrane staining observed in more than 10% of the tumor cells. Areas that were poorly preserved, crushed, folded, or retracted were specifically avoided. Scores of 2+ and 3+ were considered positive for Her-2/neu. ⁽²⁷⁾

2. Ki67:

For Ki67, at least 1000 tumor cells (×400 magnification) from the most immunopositive regions , and the percentage of positive cells (labeling index, LI) was calculated. Each slide was given a value composed of

the sum of staining intensity and the proportion of the stained cells. This proportion was graded as follows: 0 for 0-10% of tumor cells stained, 1 for 11-25% of cells stained, 2 for 26-50% of cells stained and 3 for >50% of cells stained. Staining intensity was graded as follows: 1 for light yellow, 2 for dark yellow and 3 for brown. The final staining quantification value was as follows: 0 for negative (1-2), 1+ for mild (3), 2+ for moderate (4), and 3+ for strong (5-6).⁽²⁸⁾

II. Results

3.1 Age Distribution of the sample:

The mean age of patients was 58.72 ± 1.6 (SEM) years, with a range of (30-80) years (Figure 3.1)

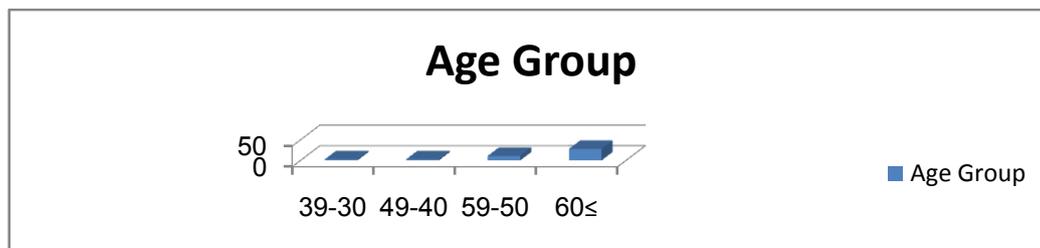


Figure 3.1 Distribution of urothelial carcinoma cases according to age groups of patients

3.2 Gender Distribution:

Gender distribution of urothelial carcinoma cases showed that 33/45 (73.3%) of cases were males compared with 12/45 (26.7%) females cases, with a male to female ratio of 2.74 : 1.

3.3 Distribution Of Cases According To WHO 2004 Grading System Of Urothelial Carcinoma

Histological examination of Hematoxylin and Eosin stain (H&E) sections confirmed that 20/45 (44.4%) cases were of low grade (Figure 3.2)

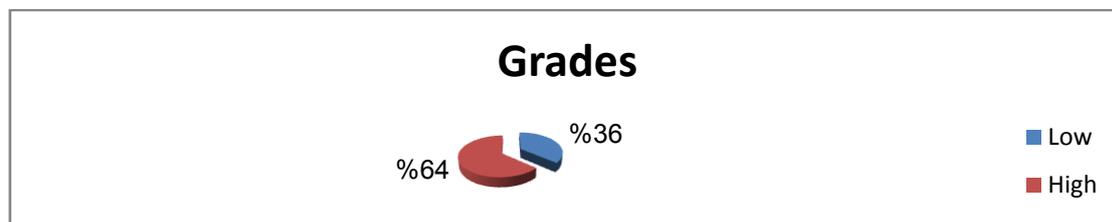


Figure 3.2 showing distribution of urothelial carcinoma cases according to WHO 2004 grading.

3.4 Ki-67 And Her2/Neu Immunohistochemical Expression In Correlation With The Age Of Urothelial Carcinoma Patients

There was no significant correlation between the immunohistochemical expression of Ki-67 and Her2/neu and the age, the P value \square 0.05

3.5 Ki-67 And Her2/Neu Immunohistochemical Expression In Correlation With The Gender Of Urothelial Carcinoma Patients

There was no significant correlation between the immunohistochemical expression of Ki-67 and Her2/neu and the gender, the P value \square 0.05

3.6 Ki-67 And Her2/Neu Immunohistochemical Expression In Correlation With The Grade Of Urothelial Carcinoma Patients

There was a significant correlation between expression of both Ki-67 and Her2/neu and the WHO 2004 grading system of the tumor. This correlation was highly significant for Ki-67 marker (p value 0.014), in which 21/25 (84%) high grade cases show positive immunohistochemical Ki-67 expression, while 10/20 (50%) of low grade show positive immunohistochemical Ki-67 expression (Table 3.1)

This correlation was also significant regarding the immunohistochemical expression of Her2/neu (p value 0.015), in which there were 20/25 (80%) of high grade carcinoma cases show positivity to immunohistochemical expression of Her2/neu marker, while 9/20 (45%) of low grade show positivity to immunohistochemical expression of Her2/neu marker (Table 3.1)

(Table3.1) Ki-67 And Her2/Neu Immunohistochemical Expression In Correlation With The Grade Of Urothelial Carcinoma Patient

	Ki-67Positivity		P	Her2/neuPositivity		P
	Low	High		Low	High	
Negative	10	4	0.014*Significant	11	5	0.015*Significant
Positive	10	21		9	20	
Total	20	25		20	25	

3.7 Ki-67 And Her2/Neu Immunohistochemical Expression In Correlation With The AJCC T Stage Of Urothelial Carcinoma Patients

Regarding Ki-67 and Her2/neu ; no significant correlation was found between Ki-67 and Her2/neu expression with the AJCC T stage of urothelial carcinoma patients(p value 0.238) (p value 0.429) respectively.

3.8 Immunohistochemical Scoring Expression Of Ki-67 In Correlation With The WHO2004 Grade Of Urothelial Carcinoma

There was a significant correlation between expression of Ki-67 and the WHO2004 grading system of the tumor(p value 0.013) .

Score+1 was present in (4/10)(40%) of high grade urothelial carcinoma cases , score+2was seen in(6/8)(75%) of high grade urothelial carcinoma cases, while score+3 was seen in (11/13)(84.6%)(Figure3.3 and 3.4)

On the other hand, there was high percentage of negative Ki-67 immuno staining regarding low grade urothelial carcinoma cases 10/14(71.42),score+1 seen in 6/10(60%) of low grad,score+2 seen in 2/8(25%) of low grade,while score+3 seen in 2/13(15.3%) of low grade(Table3.2)

Table3.2: immunohistochemical expression score of the Ki-67 in correlation with the WHO2004 grade of urothelial carcinoma cases

		Ki-67 Score				Total	P
		0	1	2	3		
Grade	Low	10	6	2	2	20	0.013* Significant
	High	4	4	6	11		
Total		14	10	8	13	45	

3.9 Immunohistochemical Scoring Expression Of Her2/Neu In Correlation With The WHO2004 Grade Of Urothelial Carcinoma

There was a significant correlation between expression of Her2/neu and the WHO2004 grading system of the tumor(p value 0.018) . Score+1 was present in (3/13)(23%) of high grade urothelial carcinoma cases , score+2was seen in(5/10)(50%) of high grade urothelial carcinoma cases, while score+3 was seen in (15/19)(78.94%)(Figure3.6).On the other hand, score+1 was seen in 10/13(76.9%) of low grade (Figure 3.5) ,score+2 seen in 5/10(50%) of low grade,while score+3 seen in 4/19(21%) of low grade(Table3.3)

(Table3.3) immunohistochemical expression score of the Her2/neu in correlation with the WHO2004 grade of urothelial carcinoma

		Her2/neu Score				Total	P
		0	1	2	3		
Grade	Low	1	10	5	4	20	0.018* Significant
	High	2	3	5	15		
Total		3	13	10	19	45	

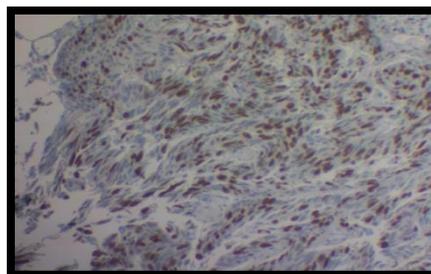


Figure3.3 High grade urothelial carcinoma(pT1) showing score+3 positive immune reaction for Ki-67 marker (×10).

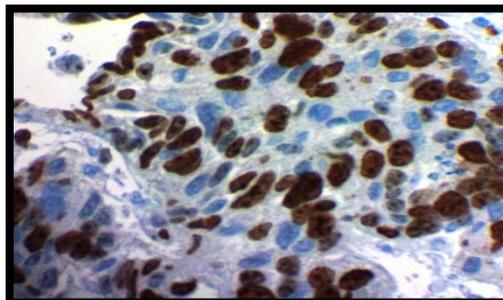


Figure3.4 High grade urothelial carcinoma(pT1) showing score+3 positive nuclear immune reaction for Ki-67 marker (×40).

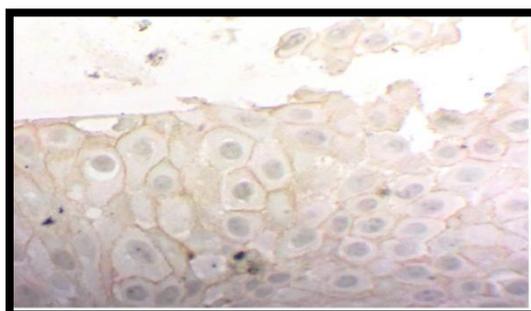


Figure3.5 low grade urothelial carcinoma(pTa) showing partial faint membranous (score+1)positive immune reaction for Her2/neu marker (×40).

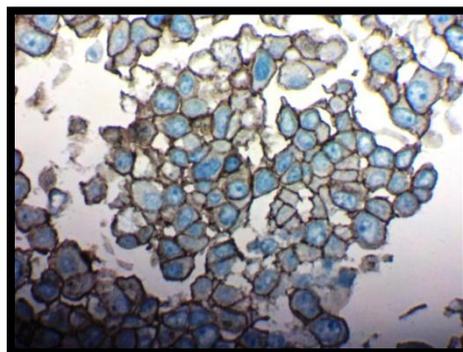


Figure3.6 High grade urothelial carcinoma(pT1) showing stronge membranous (score+3) positive immune reaction for Her2/neu marker (×40).

III. Discussion

4.1 Assessment of Her2/neu Immunohistochemical expression in urothelial carcinoma of the bladder:

The present study revealed a significant correlation between the grade of urothelial carcinoma cases and Her2/neu expression of (p value was 0.015)(Table 3.7), in which Her2/neu was over expressed immunohistochemically in high grade urothelial carcinoma than in low grade, and these results were supported by studies done by (*ElMoneim HMA and Wülfing C et al.*)^(23,29), who both found that immunohistochemical expression of Her2/neu was directly proportional with the grade of urothelial carcinoma .

4.2. Assessment of Ki-67 Immunohistochemical expression in urothelial carcinoma of the bladder:

In the present study, a highly significant correlation between Ki-67 expression and 2004 grading system of urothelial carcinoma cases was found (p value 0.014) (Table 3.7), in which HGPUCs exhibited more extensive Ki-67 expression than LGPUCs, these findings are supported by (*Gönül II*) and (*Stepan A*).^(30,31)

References

- [1]. Jamal A, Tiwari RC and Murray T. Cancer statistics . CA Cancer J Clin 2004, 54: 8-29.
- [2]. 2010 Results of Iraqi Cancer Registry. 17th ed. Baghdad, Iraq: Cancer Board, Ministry of Health; 2012.
- [3]. Kumar V, Abbas AK, and Fausto N, 2010, Robbins and Cotran, pathologic basis of disease, 8th ed, Elsevier, Philadelphia USA, p976-980

- [4]. Parkin DM. The global burden of urinary bladder cancer. *Scand J Urol Nephrol Suppl.* 2008; 218: 12_20.
- [5]. Ploeg M, Aben KK, Kiemeny LA. The present and future burden of urinary bladder cancer in the world. *World J Urol.* 2009; 27: 289_93.
- [6]. Epstein JI. The lower urinary tract and male genital system. In: Kumar V, Abbas AK, Fausto N, eds. *Robbins and Cotran Pathologic Basis of Disease.* 7th ed. Philadelphia: Saunders; 2004. p. 1023-58.
- [7]. Parkin DM, Bray FI, Ferlay J, et al. *Global Cancer Statistics, 2002.* *CA Cancer J Clin.* 2005;55:74-108.
- [8]. Kassouf W, Black PC, Tuziak T, Bondaruk J, Lee S, Brown GA, et al. Distinctive expression pattern of Erb-b famil receptors signify an aggressive variant of bladder cancer. *J Urol.* 2008; 179: 353_8.
- [9]. Latif Z, Watters AD, Dunn I, Grigor KM, Underwood MA, Bartlett JM. HER2/neu overexpression in the development of muscle-invasive transitional cell carcinoma of the bladder. *Br J Cancer.* 2003; 89: 1305_9.
- [10]. Munoz JJ, Ellison LM. Upper tract urothelial neoplasms: incidence and survival during the last 2 decades. *J Urol* 2000 Nov;164(5):1523-5.
- [11]. Ploeg M, Aben KK, Kiemeny LA. The present and future burden of urinary bladder cancer in the world. *World J Urol* 2009 Jun;27(3):289-93.
- [12]. Roupřet M, Zigeuner R, Palou J, et al. European guidelines for the diagnosis and management of upper urinary tract urothelial cell carcinomas: 2011 update. *EurUrol* 2011Apr;59(4):584-94.
- [13]. American Cancer Society: *Cancer Facts and Figures 2014.* Atlanta, Ga: American Cancer Society, 2014. Available online. Last accessed May 21, 2014.
- [14]. Howlader N, Noone AM, Krapcho M, et al., eds.: *SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations).* Bethesda, Md: National Cancer Institute, 2012. Also available online. Last accessed May 21, 2014.
- [15]. Al-Azzawi S: Depleted Uranium radioactive contamination in Iraq: An overview. *Global Res.: Center for Research on Globalization;* 2006. www.globalresearch.ca.
- [16]. Basrah Cancer Research Group: *Cancer in Basrah (2005-2008)* p 33.
- [17]. Epstein JI, Reuter VE, Amin MB. *Urinary Bladder Biopsy Interpretation.* Lippincott Williams & Wilkins, Philadelphia, PA, 2004.
- [18]. Montironi R, Lopez-Beltran A. The 2004 WHO classification of bladder tumors: a summary and commentary. *Int J Surg Pathol* 2005;13:143-53.
- [19]. Sauter G, Algaba F, Amin M, et al. Tumours of the urinary system: non-invasive urothelial neoplasias. In: Eble JN, Sauter G, Epstein JI, Sesterhenn I, editors. *WHO classification of tumours of the urinary system and male genital organs.* Lyon, France: IARC Press; 2004.
- [20]. Burger, M., van der Aa M.N., van Oers, J.M., Brinkmann, A., van der Kwast, T.H. and Steyerberg, E.C. Prediction of progression of non-muscle-invasive bladder cancer by WHO 1973 and 2004 grading and by FGFR3 mutation status: a prospective study. *Eur Urol.* 2008 Oct; 54(4): 835-43.
- [21]. Olayioye MA (2001). "Update on HER-2 as a target for cancer therapy: Intracellular signaling pathways of ErbB2/HER-2 and family members". *Breast Cancer Res* 3 (6): 385-389. doi:10.1186/bcr327. PMC 138705. PMID 11737890.
- [22]. 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.
- [23]. ElMoneim HMA, Tawfik HM, El Sherbiny YM, Tawfik ER, Analysis of Her2/neu overexpression and amplification in urothelial carcinoma of the bladder associated with Cox-2 overexpression, *Int J Cancer Res,* 2011, 7(1):8-24
- [24]. Li R, Heydon K, Hammond ME, Grignon DJ, Roach M 3rd, Wolkov HB, Sandler HM, Shipley WU, Pollack A, Ki-67 staining index predicts distant metastasis and survival in locally advanced prostate cancer treated with radiotherapy: an analysis of patients in radiation therapy oncology group protocol 86-10, *Clin Cancer Res,* 2004, 10(12 Pt 1):4118-4124.
- [25]. Gönül II, Akyürek N, Dursun A, Küpeli B. Relationship of Ki67, TP53, MDM-2 and BCL-2 expressions with WHO 1973 and WHO/ISUP grades, tumor category and overall patient survival in urothelial tumors of the bladder. *Pathol Res Pract* 2008; 204:707-717.
- [26]. Quintero A, Alvarez-Kindelan J, Luque RJ, Gonzalez-Campora R, Requena MJ, Montironi R, Lopez-Beltran A, Ki-67 MIB1 labelling index and the prognosis of primary TaT1 urothelial cell carcinoma of the bladder, *J Clin Pathol,* 2006, 59(1):83-88.
- [27]. Abd El-Aty Shawkya, Amal Abd El hafeza, e, Gehan Mohamed Elosailyb, Hisham Y. Al-Matubsic, Adel Farahatd. Her-2/Neu Overexpression in Invasive Bladder Carcinoma Among a Cohort of Egyptian Patients. *World J Nephrol Urol.* 2013;2(2):70-75
- [28]. Lujia Wang, Chenchen Feng, Guanxiong Ding, Zhongwen Zhou, Haowen Jiang, Zhong Wu. Relationship of TP53 and Ki67 expression in bladder cancer under WHO 2004 classification. *Jbuon* 2013; 18(2): 420-424
- [29]. Wülfing C, von Struensee D, Bierer S, Bögemann M, Hertle L, Eltze E, Expression of Her2/neu in locally advanced bladder cancer: implication for a molecular targeted therapy, *Aktuelle Urol,* 2005, 36(5):423-429.
- [30]. Gönül II, Akyürek N, Dursun A, Küpeli B. Relationship of Ki67, TP53, MDM-2 and BCL-2 expressions with WHO 1973 and WHO/ISUP grades, tumor category and overall patient survival in urothelial tumors of the bladder. *Pathol Res Pract* 2008; 204:707-717.
- [31]. Stepan A, Simionescu C, Mărgăritescu C, Ciurea R, P16,c-erbB2 and Ki67 immunoexpression in urothelial carcinomas of the bladder, *Rom J Morphol Embryol,* 2011, 52(2):653-658.