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The role of CISH method in detection of HER2 gene mutation in the transitional cell carcinoma of urinary bladder

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Abstract: Objectives: Evaluate the CISH method in detection HER2 amplification and to study the correlation clinicopathological data (age, sex, grade, and stage) of patients with of transitional cell carcinoma urinary bladder with HER2 gene amplification.
Design and Methods: This study is included 50 patient with bladder cancer (male 35 and female 15) with additional 20 control group (10 cases is positive control and 10 cases is negative control). 50 patients who are diagnosed transitional cell carcinoma of bladder and their biopsies of bladder (taken from endoscopic biopsy or cystectomy). Then, paraffin-embedding procedure was prepared on tissues biopsy and then, hematoxylin and eosin staining (H. and E. staining) procedure was done. Finally, manual CISH is done on both control cases(positive and negative cases) and bladder cancer blocks. This CISH method detect her2 amplification according to ASCO/CAP/2013 scoring system.
Results: The result showed that age group 61-70 years is the highest positive HER2 amplification (64.7%) among other age groups 51-60 years (11.8%) and 71-80 years old (23.5%), there is a significant association between these age groups with HER2 amplification , $p \leq 0.05$. Sex group displayed that 52.9% of male and 47.1% of female who had positive HER2 amplification and there is no significant association between sex groups with HER2 amplification, $p > 0.05$.Also, positive HER2 amplification is highest in grade 3 (88.2%) of transitional cell carcinoma when compared to grade 2 (11.8) and grade 1 (0%) and there is significant association between cancer grading system with HER2 amplification, $p \leq 0.05$. T2 and T1 of staging cancer system showed 82.4% and 17.6% positive HER2 amplification respectively with a significant correlation between them. Also, other result that the histological types of transitional cell carcinoma revealed 88.2% and 11.8% (positive HER2 amplification) of papillary and flat shape respectively with a significant correlation between them.
Conclusion: HER-2\neu amplification was significant correlated with the high grade and high stage of bladder carcinoma indicating that HER-2\neu positive tumors are biologically aggressive and associated with bad prognosis.
Key words: transitional cell carcinoma of urinary bladder, HER2 gene, CISH method.

Introduction:

The urinary bladder cancers are one of common cancer of the urinary tract. It was reported that oxidative stress status is being play a very important roles in development of carcinogenesis[(1)].The urinary

bladder cancer is regarding as second and fourth most common cancer in male for Iraq and United State America (USA) respectively, and as tenth and eleventh most common cancer for female in both Iraq and USA respectively [2,3]. The incidence is 9/100,000 for male and for female is 2/100,000 (according to world-wide age standardized 2008 data[4] .

Urothelial Carcinoma is epithelial tumor of urinary bladder (95% of urinary bladder tumor) and it shows wide ranging of tumor that it begin from papilloma and carcinoma in situ to invasive cancer . The patients with urinary bladder cancer that presented in non-muscle invasive type (75%-80%) or muscle invasive type (20%-25%)[5].

In general, the urinary bladder cancer incidence rises with increasing the age, mostly it occurs over the age 65 years old [6].

The tobacco smoking regard a major risk factor because it represents about 50% and 35% of urinary bladder cancerin the male and female respectively[7], Radiation, Chronic cystitis, Schistosomiasis[8].

Painless hematuria is the most important symptom of the cancer, it presented with 20% of gross hematuria and 5% of microscopic hematuria[9]. Fluorescence cystoscopy that combined with Optical coherence tomography show 97.5% of sensitivity and 97.9% for specificity[10].

The NMIBC shows two groups, the first one (represent about 70%) has high degree of recurrence but 5-year survival rate is more than 80% and other group (represent about 30%) has high degree of progression with aggressive and worsening prognosis[11].

NMIBC can convert to MIBC within 3 year survival rate about 37%. The 5-year survival rate for the tumor that invade urinary bladder wall is 55%-65% and for the tumor that metastasis out the urinary bladder is about 18%[12].

Epidermal growth factor receptor (EGFR or called HER or type I ErbB tyrosine kinase receptors) family composed of four receptor member which include HER1 (ErbB1 or EGFR), HER2/neu (ErbB2 or C-ErbB2) , ErbB3 (HER3), and ErbB4 (HER4) [13].

HER2/neu protein is glycoprotein produced by HER2 gene which lies in 17q12-21 (long arm of chromosome 17) . HER2 protein is glycoprotein (it is a transmembrane receptor and its molecular weight is 185 KD) and produced by HER2 gene which lies in 17q12-21 (long arm of chromosome 17). This glycoprotein find normally in lung, breast, urinary bladder, prostate and stomach, and its cytoplasmic portion have enzymatic function (tyrosine kinase activity). This receptor is responsible cellular control of cell differentiation, metabolism, cell survival normal cell growing, proliferation, blood vessel formation (angiogenesis)[14], and inhibition of apoptosis[15].

HER2/neu protein over expression is measured by IHC when the result of IHC is equivocal for HER2/neu protein (+ 2). Then, HER2 gene amplification is measured by using an accurate and more practical CISH method [16].

Methods

This study (retrospective (30 cases) and prospective (20 cases)) was conducted in Al-Hilla Surgical Teaching Hospital and the period of study is extended from October /2015 to August/2016. clinical data of patients include age, sex, clinical features, and type of bladder biopsy (endoscopic resection or cystectomy) are taken with paraffin bolcks. The practical side of the study was done in laboratory of department of pathology / College of Medicine/Babylon university. This study is included 50 patient with bladder cancer (male 35 and female 15) with 20 control group (10 cases is positive control and 10 cases is negative control) .

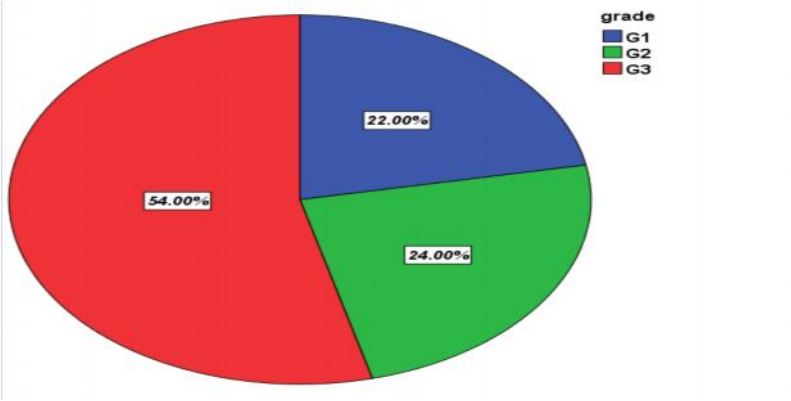
First group includes 50 patients who are diagnosed with transitional cell carcinoma of urinary bladder with mean age ± SD (66.04 ± 10.148) years old ranging between 42-83 years old. Second group is a positive control cases (10 paraffin tissue sections of breast cancer patients) that are treated by immnohistochemical procedure for her2/neu protein detection and they shows score +3 (her2/neu positive) which is run with each CISH procedure. Third group is negative control group (10 paraffin tissue sections of breast cancer patients)

that are treated by immnohistochemical procedure and they shows score +1 (her2/neu positive) or her2/neu negative which is run with each CISH procedure.

50 patients who are diagnosed transitional cell carcinoma of bladder and their biopsies of bladder (taken from endoscopic biopsy or cystectomy). Then, paraffin-embedding procedure was prepared on tissues biopsy and then, hematoxylin and eosin staining (H. and E. staining) procedure was done. Finally, manual CISH is done on both control cases(positive and negative cases) and bladder cancer blocks. This CISH method detect her2 amplification according to ASCO/CAP/2013 scoring system.

Results

The distribution of patients by grading of urothelial carcinoma of bladder. (54%) of patients presented with grade three . Meanwhile, only (22%) and (24%) of patients were presented with grade one and grade two respectively as shown in figure (1) .



Figure(1): Distribution of Patients by the grading of urothelial carcinoma of urinary bladder

The patients in the study were divided according to the age (including only positive HER2/neu amplification age group) into three groups (51-60, 61-70 and 71-80 years old). 64.71% is the highest percentage of positive HER2/neu amplification age group was observed among the patients aged 61-70 years.P value ≤0.05 is significant for all age groups(P value =0.019).

Positive HER2/neu amplification was observed in both sexes where it was found in 52.9% of males and 47.1 % of females (Positive HER2/neu for both sex), while negative HER2/neu amplification was found in both sexes where it was found in 78.8% of males and 21.2 % of females(negative HER2/neu for both sex). The difference between them was statistically (P > 0.05) insignificant (P value =0.059).

The grading of urothelial carcinoma(including both positive and negative HER2/neu amplification cases) is divided into three grade . 88.23% is the highest percentage of positive HER2/neu amplification was observed among the patients with grade three. While 11.76% and 0% amplification were observed among the patients with grade two and grade one respectively .P value ≤0.05 is significant for all grading groups(P value =0.02) .

The staging (T) of urothelial carcinoma(including both positive and negative HER2/neu amplification cases) into two main types, including T1 (NMIBC) and T2(MIBC) that depending on whether there is muscle invasion or not . 82.4% is the highest percentage of positive HER2/neu amplification was observed among the patients T2. While 17.6% positive HER2/neu amplification was observed among the patients with T1 .P value ≤0.05 is significant for all grading groups(P value =0.02) .

The histological types of urothelial carcinoma(including only positive HER2/neu amplification cases) into two main types, including flat and papillary shape . 88.2% is the highest percentage of positive HER2/neu amplification was observed among the patients papillary histological type. While 11.8% positive HER2/neu

amplification was observed among the patients with flat histological types .P value ≤0.05 is significant for all grading groups(P value =0.02) .

A **B**

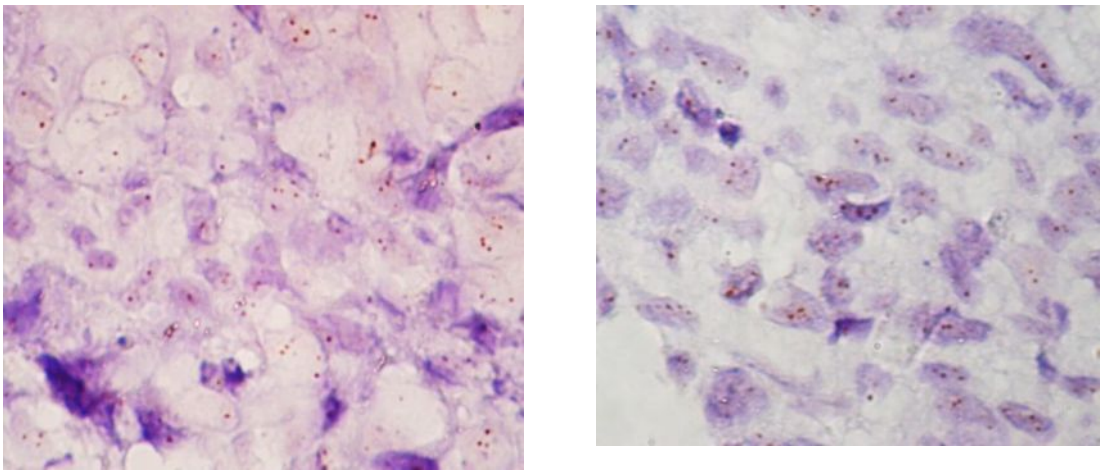


Figure (2): Section of Urothelial carcinoma (grade three-MIBC): (A)non-amplified tumor cells: two to three brown signals (HER2 gene) present in each nucleus at 40 X, and (B) low-amplified tumor cells: four (blue arrow) brown signals (HER2 gene) present in some nucleus at 40 X.

Table (1):Association of clinicopathological data with HER2 gene amplification

Variable	HER2 gene amplification (%)	*Pvalues*
Age		
51-60 years	2 (11.8)	**0.019***
61-70 years	11(64.7)	
71-80 years	4(23.5)	
Sex		
Male	9(52.9)	0.059
Female	8(47.1)	
Tumor grade		
Grade I	0(0%)	**0.02***
Grade II	2(11.8)	
Grade III	15(88.2)	
Stage(T)		
T1	3(17.6)	
T2	14(82.4)	**0.02***
Histological type		
Papillary shape	15(88.2)	**0.02***
Flat shape	2(11.8)	

*p value ≤ 0.05 is significant

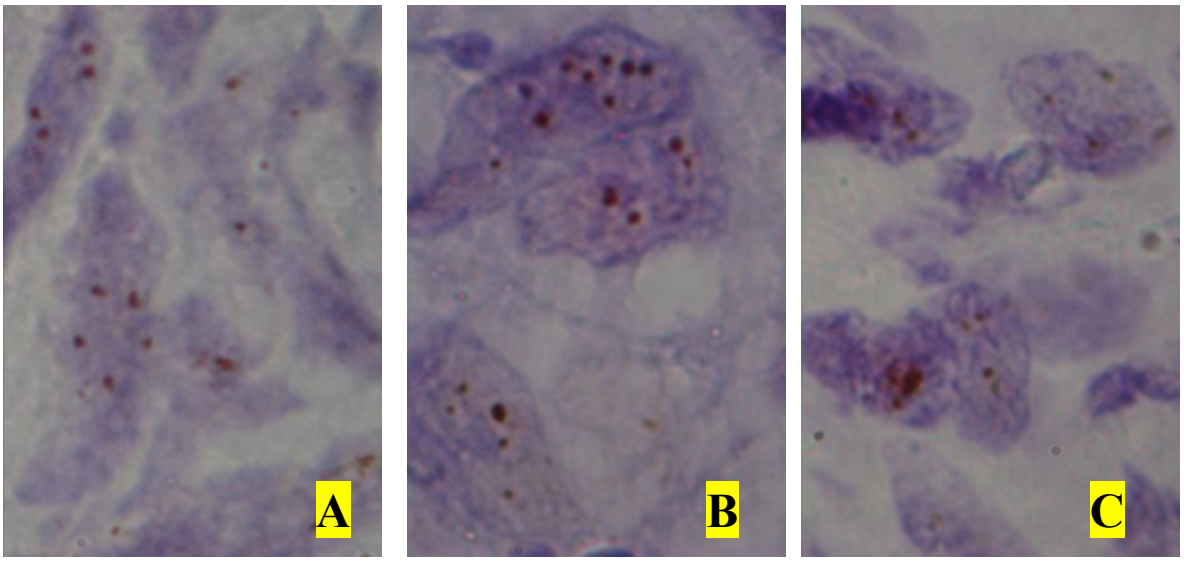


Figure (3): Section of Urothelial carcinoma (grade three-MIBC) : (A) low-amplified tumor cells: four brown signals (HER2 gene) present in each nucleus , (B) high amplified tumor cells: more than six brown signals (HER2 gene) present in each nucleus , and (C) shows high amplified tumor cells: cluster (HER2 gene) present in nucleus at 100 X.

Discussion

In present study, 70% and 30% of the urothelial carcinoma found in male and female groups respectively. This is slightly differs from Iraqi Cancer Registry Team (2011) that documented 74.5% and 25.5% found in male and female groups respectively[1] . Also, this results is similar to the study in which 70.6% and 29.4% for the male and female groups respectively [17] .

In our study, mean age is 66.04 years old, this is slightly differs to study (**HadeelA.Kerbel*et al***) in which the mean age 59.5 years old[18] .

Also, this study shows 22%, 24%, and 54% for grade I, II, and III respectively and this outcomes is slightly different from study (**HadeelA.Kerbel*et al***) that presented with 33.3% , 30% and 36.7% for grade I, II, and III respectively[18] .

The positive Her2 gene amplification in the urothelial carcinoma in of urinary bladder in our study is 34% and this is differs from other studies that shows 10% of the neoplasm [19] , 4-32% [20] and 3%-41% [21] .

The different in these percentage in our study in one side and also, the different between these other studies in other side is due to HER2 amplification is different among different population regarding ethinic and geographical variations and that will increase possibility of hypothesis in which there is an important etiological heterogeneity in bladder cancer which is responsible for this difference [22] .

Also, **Sauter*et al*** refer to obvious heterogeneity of HER2 gene in bladder cancer due to wide-ranging of number of copy gene within that tumor[23] .

In present study shows that positive HER2 gene amplification are 11.8%, 64.7%, and 23.5% for age group 51-60, 61-70, and 71-80 years old respectively, this high percentage in age group (61-70 years old) is associated with high grade and high stage .

In this recent study shows that HER2 gene amplification are 47.1% and 52.9% for female and male respectively , this indicate that type and grade of urothelial carcinoma is not related to sex group.

our study shows that positive HER2 amplification are 0%, 11.8%, and 88.2% for grade I, II and III respectively and this results is different from study which shows 0%, 17.6%, and 82.4% for grade I, II and III respectively[17] .

Also, **Chen *et al.*** who demonstrate the association between HER2 amplification and high grade NMIBC by using IHC and FISH procedures [(24)], and other studies shows that is more association between HER2 amplification and high grade, invasive and recurrent tumor [(25)]. This is agree with our study.

In recent study shows that positive HER2 amplification are 17.6% and 82.4% for stage T1and T2 respectively and other studies gives different in which the result is the 27.3% , 63.7% and 9% for T1 ,T2 and Ta respectively[(26)] . This different is due to number different in sample size between our study and other studies.

Other study shows HER2 amplification is associated with higher stage (worsened stage) and shorter survival [(27)].

Lipponen et al demonstrate that HER2 amplification is correlated with higher stage and grade with shorter survival rate[(28)].

Bolenz et al is also documented that HER2 amplification (in MIBC cases) is associated with twice risk in recurrence and mortality in when they are adjusted for tumor stage, grade, lymph nodes metastases and lymphovascular invasion [(29)].

Others reported that HER2 amplification is correlated with higher grade and stage that is possibly result from genetic effect (HER2 amplification) on tumor differentiation[(30)]. HER2 gene has enzymatic function (tyrosine kinase activity) responsible cellular control of cell differentiation, metabolism, cell survival normal cell growing, proliferation, blood vessel formation (angiogenesis)[(14)], and inhibition of apoptosis[(15)].

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