Correlation of HER-2/neu Oncogene Expression And Grades of Transitional Cell Carcinoma of Bladder

Dr. Maather Baqer Hussein Al-Harmooshy / Msc general pathology
Department of pathology/ Faculty of Medicine/ Al-Qaddissia University

الخلاصة:-
البحث عن التعبير المناعي النسيجي الكيميائي لجين أل HER-2/neu في خلايا سرطان المثانة الانتقالي ومعرفة ترابط هذا التغير مع درجة تمايز الورم. تمت دراسة ٥٠ عينة من سرطان المثانة الانتقالي في مختبرات مستشفى الديوانية التعليمي للفترة منذ بداية كانون الثاني ٢٠٠٦ وحتى كانون الأول ٢٠١٠، تراوحت أعمارهم بين ٣٣ و٨٢ سنه مع معدل عمر ٥٧.٥ سنه،٣٢ ذكر و١٨ أنثى، صنفت العينات حسب درجة التمايز إلى ٩ عينات من الدرجة الأولى ٢٤ عينة من الدرجة الثانية و١٧ عينة من الدرجة الثالثة على التوالي. أظهرت الدراسة المناعية النسيجية أن تعبير أل- HER-2/neuكان موجبا في ١٩ عينة (٣٨%) من سرطان المثانة الانتقالي، وكان أكثر في العينات ذات التمايز الضعيف (الدرجة الثالثة) عنه في العينات ذات التمايز القوي والمتوسط (الدرجة الأولى والثانية). كما انه توجد علاقة واضحة لتعبير ا لHER-2/neu مع درجة التمايز (قيمة ألفا < ٠.٠٥). مما يدل على أن جين أل-HER-2/neu يلعب دور أساسي في تقييم حالة سرطان المثانة الانتقالي المستقبلية وانتشاره وتحديد أهمية الاستجابة للعلاج.

Abstract:-
This study was conducted to estimate the overexpression of HER-2\neu oncoprotein in human transitional cell carcinoma of bladder and to show its possible correlation to the specific pathological parameters like grade of tumor. We evaluated the available tissue blocks of 50 patients with transitional cell carcinoma who had referred to Al-diwaniya Hospital/ department of pathology in a period between January 2006 and December 2010, 32 cases were male and 18 cases were female. The mean age of the patients was 57.5 years (range from 33 to 82 years). The cases were classified according to the grading system into grade I, II, and III in 9, 24 and 17 cases respectively. From 50 cases of TCC, A total of 19 (38%) patients were positive for overexpression of *HER-2/neu* oncogene, 12 (63.2%) cases of them with predominant membranous and cytoplasmic staining and 7 (36.8%) cases of them with cytoplasmic staining , there was no pure membranous staining. High histological grades of the TCC were associated with increased expression of *HER-2/neu*, There was 1 (11%) case of *HER-2/neu*-positive with a grade I tumor, 7 (29%) with grade II, and 11 (63.7%) with grade III (*P* =.002) There was a significant relationship in HER-2/neu over expression with high grade of TCC.

Aim of the study:
The aim of this study was to evaluate the relationship between *HER-2/neu* oncogene expression and grade of transitional cell carcinoma (TCC) of the bladder.

Key words:- Transitional cell carcinoma , HER-2/neu oncogene.

Introduction:-
Recent scientific attention has focused on the role of growth factors in the progression of cancer. **HER2/neu** (also known as **ErbB-2**) stands for "Human Epidermal growth factor Receptor 2" and It is a member of the ErbB protein family, more commonly known as the epidermal growth factor receptor family. HER2/neu has also been

designated as **CD340** (cluster of differentiation 340) and **p185**. It is encoded by the *ERBB2* gene[(1)].

HER2 is a cell membrane surface-bound receptor of a 185 kDa transmambrane tyrosine kinase belonging the epidermal growth factor receptor family which comprises four homologous receptors Erb-1 (EGFR,HER1) , Erb-2 (HER2/neu), Erb-3 (HER3) , and Erb-4 (HER4)[(2)] that is overexpressed on the cell surface of approximately 25% to 30% of breast cancers[(1)] . Activation of c-erb2 oncoprotein , either by homo-or hetrodimerization , trigger intracellular signaling events leading to cell growth , differentiation and survival[(2)] . It is encoded within the genome by HER2/neu, a known proto-oncogene. it is thought to be an orphan receptor, with none of the EGF family of ligands able to activate it. However, ErbB receptors dimerise on ligand binding, and it is the preferential dimerisation partner of other members of the ErbB family.[1] The *HER2* gene is located at the long arm of human chromosome 17(17q21-q22)[(2)].

Overexpression of *HER-2/neu* has been associated with some different types of human cancers**;** in one study it is overexpressed in 13/59 cases of breast carcinoma , 8/29 cases of pulmonary adenocarcinomas , 10/58 cases of colorectal carcinoma , 6/56 cases of pulmonary squamous carcinoma and 7/62 cases of gastric adenocarcinoma [**(4)**] .15/112 cases of endomaterial carcinoma [(5)] in other study [(6)] 101/177 of transitional cell carcinoma of the urinary bladder showed overexpression of HER2 oncoprotein .

Bladder cancer is a common tumour. Over 10,000 new bladder cancers are diagnosed annually in the UK. This equates to 4% of all cancers in Britain. Bladder cancer accounts for nearly 5000 deaths each year in the UK. There are over 60,000 newly diagnosed cases in the USA with 13,000 deaths due to it each year. It is estimated that 67160 new cases of TCC of the bladder and 13 750 deaths will occur in 2007 in the United States due to this disease. For local tumors and distant metastases, the 5-year survival rates are 48% and 6%, respectively[(7)] . In men it is the fourth most common cancer after prostate, lungs, and colorectal malignancy accounting for 6.2% of all cancer cases. In women it is the eighth most common cancer accounting for 2.5% of all cancer cases[(8)]. Over the last 25 years the mortality rate for bladder cancer has been falling[(7)].

Life expectancy will increase in patients with TCC if a correct and early diagnosis is made. Several factors involve in determination of prognosis and selection of the treatment. A known dependent factor is the grade of the tumor[(8)]. Other factors such as growth rate, patient's age, and tumor aggressiveness are also important[(9)]. However, they may be inadequate in determination of the prognosis and therefore, other factors such as oncoproteins can be helpful[(4)]. The clinical course of bladder carcinoma carries a broad spectrum of aggressiveness and risk. Low-grade, superficial bladder carcinoma have minimal risk of progression to death; however, high-grade with muscle-invasive carcinoma are often lethal[(7,10)].

Material and method :-

We evaluated the available tissue blocks of 50 patients with bladder TCC who had referred to Al-Diwaniya Hospital / department of pathology in a period from January 2006 and December 2010. Three micrometer thick sections were prepared from paraffin-embedded tissue blocks and stained by hematoxylin-eosin method. Tumor grade was then determined using the World Health Organization/ International Society of Urological Pathologists criteria[(10)] by a single pathologist blinded to the records of the patients. A manual avidine-biotin-peroxidase complex procedure was used in the immunohistochemical analysis (Dako Cytomation, Copenhagen, Denmark);the

expression is classified into cytoplsmic staining and membranous and cytoplasmic staining.

The membrane staining intensity and pattern were considered for scoring according to the Food and Drug Administration approved criteria[11]. Which are as the fallowing Zero = no staining or membrane staining observed in less than 10% of the tumor cells. 1+ = partial membrane staining in more than 10% of the tumor cells and membrane staining not circumferential.

2+ = circumferential weak to moderate staining observed in more than 10% of the tumor cells

3+ = strong circumferential membrane staining observed in more than 10% of the tumor cells.

Data were analyzed using the SPSS software version 17 and chi-square was used .

Result :-

50 cases of TCC were included in this study, 32 (64%) were male and 18 (36%) were female. The mean age of the patients was 57.5 years (range, 33 to 82 years). The cases were classified into grade I, II, and III in 9 (18%), 24 (48%), and 17 (34%) cases, respectively [table 1]. A total of 19 (38%) patients were positive for over expression of *HER- 2/neu* oncogene. 12 (63.2%) cases had membranous (predominant) and cytoplasmic staining and 7 (36.8%) cases had cytoplasmic staining, There was no case with pure membranous staining and 73.7%of the positive cases was with strong positive 3+ and with score 2 in 10.5% and in score 1 in 15.8% [table 2]. High histological grades of the TCC were associated with increased expression of *HER-2/neu*. There were 1 (11.1%) case of *HER-2/neu* positive with a grade I tumor, 7 (29.1%) with grade II, and 11 (64.7%) with grade III with significant relationship (P = .002) [table 2]. The percent of membranous staining was increased from 57.1% in grade II to 81.8% in grade III [Table 3].

Table1:- Clinicopathologic characteristics of TCC cases.

Clinicopathological characteristic		
Sex	Male	32 (64%)
	Female	18 (36%)
Age	≥ 50	30 (60%)
	< 50	20 (40%)
Grade	I (Well differentiated)	9 (18%)
	II (Moderately differentiated)	24 (48%)
	III (Poorly differentiated)	17 (34%)

Table 2:- HER-2/neu expression pattern in TCC cases.

Staining	**Positive**	**19 (38%)**
	Negative	**31 (62?%)**
Pattern	**Cytoplasmic**	**7 (36.8%)**
	Membranous +Cytoplasmic	**12 (63.2%)**
Percent of staining	**+1**	**3 (15.8%)**
	+2	**2 (10.5%)**
	+3	**14 (73.7%)**

Table 3:- HER-2/neu expression pattern in relation to grades of TCC.

		Grade I	**Grade II**	**Grade III**
Staining	**Positive**	**1 (11.1%)**	**7 (29.1%)**	**11 (64.7%)**
	Negative	**8 (88.9%)**	**17 (70.9%)**	**6 (35.3%)**
Pattern	**Cytoplasmic**	**0**	**3 (42.9%)**	**4 (36.4%)**
	Membranous +Cytoplasmic	**1 (100%)**	**4 (57.1%)**	**7 (63.6%)**
Percent of staining	**+1**	**0**	**1 (14.2%)**	**2 (18.2%)**
	+2	**0**	**1(14.2%)**	**1 (9%)**
	+3	**1 (100%)**	**5 (71.6%)**	**8 (72.7%)**

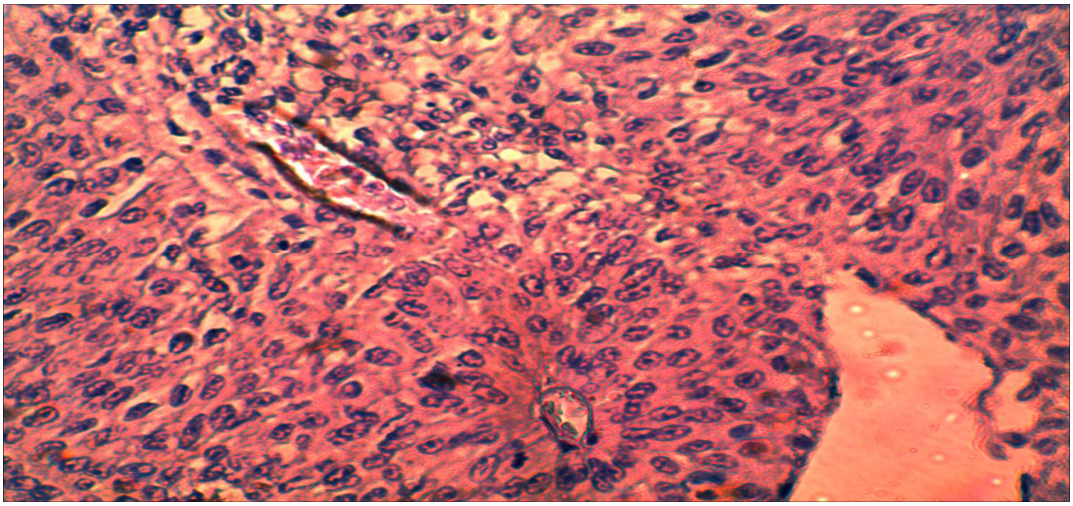

Figure 1: Color TCC, HER2 staining, negative , 40×.

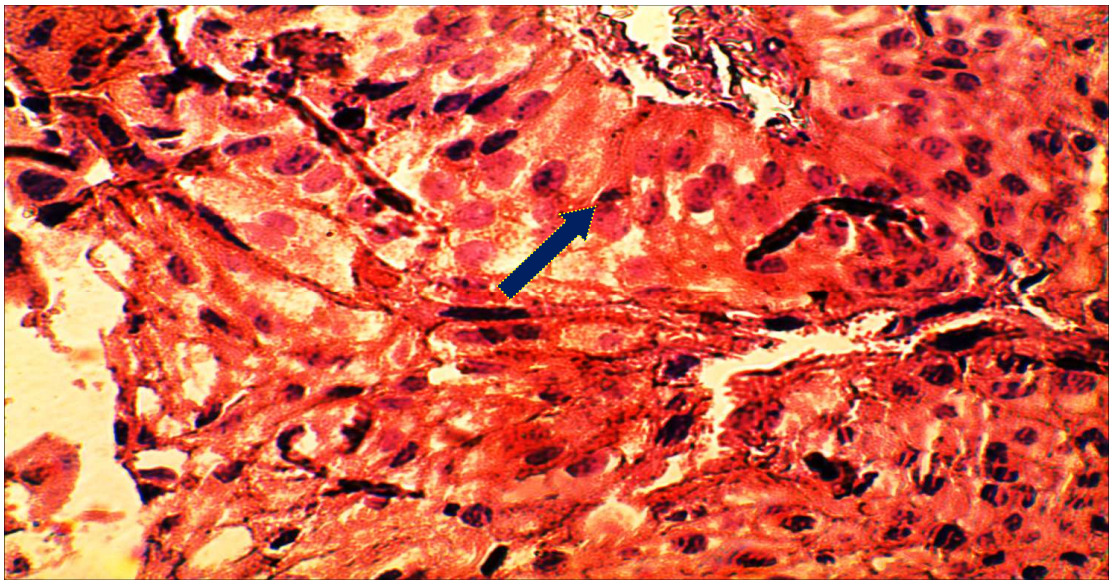

Figure 2:-Color TCC , HER2 staining, cytoplasmic , 40×

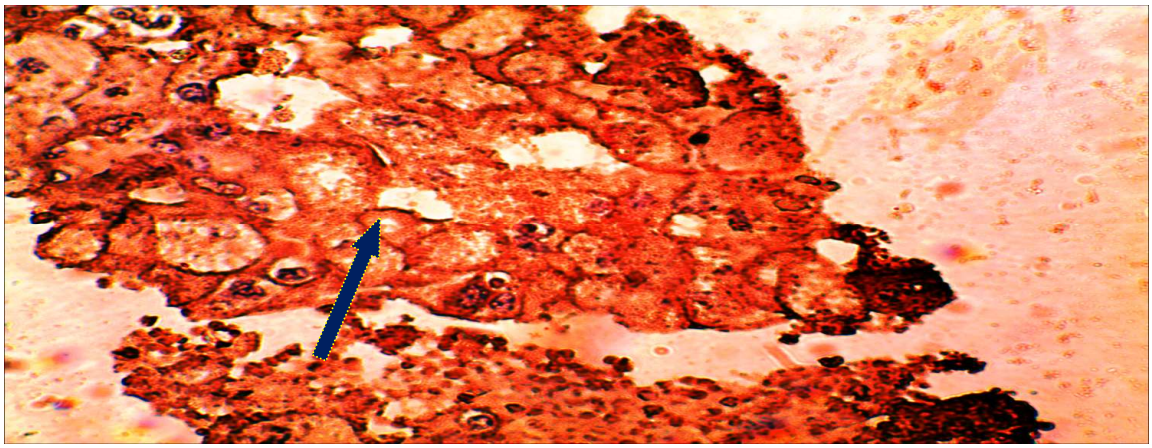

Figure 3: Color TCC , HER2 staining, membranous and cytoplasmic, 40×.

Discussion:-

The identification of molecular markers that provide an insight into the potential behavior or aggressiveness of tumors is a necessary step for the improvement of cancer treatment. The association between HER-2/neu gene amplification and poor prognosis was first determined in 1987 by Slamon *et al* on breast cancer [(12)]. In the present study, HER-2/neu over expression was seen in 38% of TCC patients .This characteristic is associated with more aggressive tumor behavior, according to our findings, higher grade of the TCC of the bladder is accompanied by *HER-2/neu* over expression with statistical significant relationship, this in agreement with Korkolopoulou P et al in 1997 that performed on a total of 106 patients, it was revealed that higher grade and stage of the TCC tumor correlated with more *HER-2/neu* overexpression[(13)]. These results also agree with the results of Mellon and colleagues and Coombs and colleagues[(14,15)].

One of the important features of HER2 staining in TCC in our study was the pattern of staining. There was membranous staining in addition to cytoplasmic staining , It means that other mutations must be responsible in TCC. On the other hand, because of more membranous staining and high percent of strong expression in higher grades , drugs that targeting HER2 may be helpful in these cases. On the study by Lipponen and his associates on 91 patients with bladder TCC showed 11 patients (12%) to be *HER-2/neu*-positive and in 4% of them, the expression was graded as moderate or severe[(16)], they found out that moderate and severe over expression of *HER-2/ neu* oncoprotein in TCC seemed to be related to a more aggressive behavior of the tumor, while low expression of this oncoprotein had no predictive value[(16)]. Although that study had some differences in the method of cellular evaluation, it was similar in other ways to ours. Also the more membranous staining in higher grades this agree with more studies on colonic carcinoma [(17,18)], but there is no current studies in TCC carcinoma revealed that . Tetu and coworkers studied low malignant potential papillary TCC tumors and found out that *HER-2/neu* expression was unremarkable in superficial bladder cancer which is compatible with our results[(19)]. In another study, 36% of cases were positive for *HER-2/neu* using the immunohistochemistry method precisely similar to our study and might confirm our results[(20)].

In the study by Latif et al , *HER-2/neu* and muscle invasiveness were evaluated in 25 patients with TCC. No significant relation was detected in the expression of this protooncogene and grade of the tumors; however, the percentage of positive cells for *HER-2/neu* was greater in invasive tumors suggesting anti-*HER-2/neu* treatment in invasive cases [(21)]. Our sample size was greater, but their method was more sensitive since they used fluorescence in situ hybridization rather than immunohistochemistry.

Conclusion :-

Analysis of HER-2/neu status in TCC carcinoma is important because it provides valuable prognostic, predictive and therapeutic information, as we did in the present study, this gene can be used in determination of the prognosis of bladder TCC and Our results showed that HER-2 over-expression correlates with aggressiveness parameters such as high histological TCC grade. And because of more prominent membranous staining in higher grades, the Herceptin therapy could be helpful in patients with TCC patients .

Recommendation :-

comprehensive research with longer follow-up period and larger sample sizes are needed for further elucidation of the role of the HER-2/neu oncogenes.

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