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The Role of c-ERBB2(Her-2) and P53 in Differentiating Low Grade from High Grade Urothelial Carcinoma of Urinary Bladder

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Abstract

Background: Urothelial carcinoma of the urinary bladder, represents 90% of all primary tumors of this organ, and one of the most common ten malignancy in Iraq and world wide. These tumors can range from low grade papillary neoplasms to less frequent more aggressive and invasive solid tumors. More than 70% of superficial tumors recur, and about one-third of the patients have tumor progression, which can affect the quality of their life HER-2\neu receptor as an important growth factor . This receptor possesses intrinsic tyrosine kinase activity and all members of this family are frequently overexpressed and \or their respective genes are amplified in human neoplastic tissue . Its overexpression has been associated with high grade and advanced stage of bladder carcinoma specially TCC. Altered expression of the p53 gene has also been associated with transitional cell carcinoma. Materials and Methods: 62 patients with TCC were included in this study and conducted in the Department of Pathology, Faculty of Medicine, Kufa University during the period from January 2012 through April 2013. EnVision method (polymer based immunohistochemistry) was used for the immunohistochemical detection of p53 and Her_2/neu. Results: HER-2\neu overexpression was found in 62.9% of bladder carcinoma , and no expression in benign bladder lesions (cystitis) was detected , (P value < 0.05) . HER-2\neu overexpression and its intensity were well correlated to grade of tumor indicating that HER-2\neu positive bladder carcinoma are biologically aggressive and detected more frequent in grade III than grade I or grade II (P value < 0.05, R = 0.0181).P53 was expressed in (66.12%) of bladder carcinoma , and no expression in benign bladder lesions (cystitis) was detected , (P value < 0.05) . P53 overexpression and its intensity were well correlated to grade of tumor indicating that P53 positive bladder carcinoma are biologically aggressive and detected more frequent in grade III than grade I or grade II (P value < 0.05, R = 0.0821). Conclusions: From the above results we concluded that p53 and Her_2/neu over-expression play an important role in pathogenesis of urothelial carcinoma evolution, as their positivity associated with higher tumor grade.

Introduction

Urothelial carcinoma, formerly known as transitional cell carcinoma is a common malignancy, representing approximately 90% of all primary tumors of this organ .[1] In Iraq it is one of the ten most common cancers according to Iraqi cancer registry of 2005.[2,3] Urothelial carcinomas range from papillary to flat, noninvasive to invasive and low grade to high grade. Low-grade carcinomas are always papillary and are rarely invasive, but they may recur after removal.[4] Tumor progression with increasing degrees of cellular atypia and anaplasia are associated with an increase in the size of the lesion with increasing tendency of invasion into deeper structures of the bladder wall.[4] Occasionally, these cancers show foci of squamous cell differentiation; only 5% of bladder cancers are true squamous cell carcinomas (SCC) . Adenocarcinomas account for less than 2% of primary bladder tumors. The urinary bladder may also be affected rarely by neuroendocrine tumors (including small cell carcinoma), carcinosarcoma, malignant lymphoma, rhabdomyosarcomas (mainly in children), and leiomyosarcoma. The latter is the most common sarcoma of the bladder.[2,4,5]. Aims of the study: to assessment of P53 and Her/2neu expression in different grades of urothelial carcinoma, correlation between the expression of both markers and the use of P53 and Her/2 as prognostic markers.

Materials and Method

This study was carried out from January 2012 through April 2013. Sixty two patients with TCC of the urinary bladder were included in this study, fifteen specimens of benign bladder lesions taken from patients with cystitis were considered as control group, The specimens were formalin-fixed, paraffin embedded tissue blocks, from these blocks, 5 micrometer-thick tissue sections were obtained and stained with hematoxylin eosin staining method and immunohistochemical Envision staining method. Scoring according to Sophia KA et al [6] at objective 40 as in P53 Scoring system 64 and HER2/neu scoring system. Statistical analysis was done with SPSS software version 18 (SPSS Inc, Chicago IL). Significant differences between non-parametric variables were done by Chi squared test and between parametric variables by independent t-test. Pearson Correlation was calculated to evaluate the relations between parameters. P values ≤ 0.05 were considered statistically significant and P values ≤ 0.01 were considered statistically higher significant.

Results

Sixty two cases of bladder carcinoma were included in this study 50(80.65%) patients were males and 12 (19.35%) were females (Table 1) , showing highly significant difference between male and female.

Table 1 :immunohistochemically expression of Her_2/neu and P53 in relation to patient gender:

Positive	Her2_neu expression		Total
	Negative		
Female	4(33.34%)	8(66.66%)	12(19.35%)
Male	35(70%)	15(30%)	50(80.65%)
Total	39(62.90%)	23(37.10%)	62(100%)
Gender	P53 expression Positive	negative	Total
Female male total	10(83.33%) 31(62%) 41(66.12%)	2(16.67%) 19(38%) 21(33.88%)	12(19.35%) 50(80.65%) 62(100%)

P>0.05 not significant

Assessment of age presentation of patients , there is a significant difference between age group (Table 2) . The intensity of immunostaining of HER-2\neu protein was assessed in relation to the grade of tumor ,and there is a significant difference between the intensity of HER-2\neu and the grade of tumor (R= 0.0181) (Table 2). The intensity of p53 was assessed in relation to the grade of tumor , and there is a significant difference between the intensity of HER_2/neu and the grade of the tumor (p <0.05) (R=0.691)

Table 2: Correlation between intensity of HER-2\neu and P53 with grade of bladder carcinoma .

Grade	HER-2\neu intensity (Score)				Total
	0	+1	+2	+3	
Grade I	6 33.33%	5 27.77%	4 22.22%	3 16.68%	18 29.05%
Grade II	5 25%	5 25%	3 15%	7 35%	20 32.25%
Grade III	1 4.16%	1 4.16%	8 33.33%	14 52.33%	24 38.70%
Total	12 19.35%	11 17.74%	15 24.19%	24 38.72%	62 100%

P<0.05 , R=0.691

Immunohistochemical analysis of HER-2\neu protein overexpression in relation to the age group of bladder carcinoma patients ,there was no relationship between the age group and HER-2\neu overexpression (P > 0.05) (Table 3) . Immunohistochemically analysis of p53 protein overexpression in relation to the age group of bladder carcinoma patients ,there was no relationship between the age group and p53 overexpression (P > 0.05) (R= - 0.025).

Table 3: Immunohistochemical expression of HER-2\neu and P53 in relation to the age group in bladder carcinoma .

Age group (years)	Immunostaining of HER-2\neu		Total
	Positive	Negative	
40-49	4 44.45%	5 55.55%	9 14.51%
50-59	12 85.72%	2 14.28%	14 22.58%
60-69	11 61.12%	7 38.88%	18 29.05%
70-79	12 63.16%	7 36.84%	19 30.64%
80-89	0 0%	2 100%	2 3.22%
Total	39 60.90%	23 37.10%	62 100%

P > 0.05 not significant

There was well correlation in expression of both markers in relation to grade of tumor (R = 0.8956) but no significant difference among these grades (P = 0.464) (Table 4).

Table 4: Correlation between Her2/neu and P53 immunostaining regarding grades of the presented bladder tumor.

Grade of tumor	Immunostaining of HER2		Immunostaining of P53	
	Positive	Negative	Positive	Negative
Grade I	7 (38.88%)	11 (61.22%)	8 (44.44%)	10 (55.55%)
Grade II	10 (50%)	10 (50%)	12 (60%)	8 (40%)
Grade III	22 (91.66%)	2 (8.33%)	21 (87.5%)	3 (12.5%)
Total	39 (62.91%)	23 (37.09%)	41 (66.12%)	21 (33.88%)

(P = 0.464, R = 0.885)

Discussion

HER-2\neu in the presented benign and malignant bladder tissue. Regarding the assessment of the control group (cystitis), all cases were negative for HER-2\ neu immunohistochemical staining this finding agrees with that reported by *Sanna E .et al.,* (2004)[8] . Truls G ,*et al*., (2005) [7] , showed high percentage of (80 %) HER-2\neu overexpression in bladder carcinoma .Wester K , *et al* ., (2002) [9], found that HER-2\neu was overexpressed in 81% of the bladder carcinoma. While Rafael E*, et al .,(* 2001) [10] , found that the HER-2\neu overexpression were 28 % of bladder carcinoma. Mohammed Reza N , *et al .,* (2004) [11]. Regarding the intensity of HER-2\ neu in correlation with TCC type of urinary bladder carcinoma the finding agreed with Vildan C, *et al* ., (2008) [12] , the majority of HER-2/*neu*–positive patients (93%) were detected by IHC, and a minority (26%) were positive by FISH or serology (23%). This is very important in the new research, in studying the role of trastuzumab, This often requires eligible patients to have either +3 overexpression by IHC or +2 expression by IHC with positive FISH results [13] . The immunohistochemical analysis of the results revealed that HER-2\neu overexpression agreed with Regina E, *et al* (2002) [15] showed that the HER-2\neu expression was significantly higher in high grade muscle invasive bladder carcinoma .Coogan CL , *et al* ., (2004) [16] , had found that overexpression of HER-2\neu was significantly increased in grade III and invasive tumors than grade I , II or superficial tumors, Gokhan Atis ,et al ., (2007)[17] had found that overexpression of HER-2\ neu increased as the grade increased , Mohammed Reza N ,*et al* ., (2004) [11] , found that the overexpression of HER-2\neu has a direct significant relationship with grade of TCC of urinary bladder .Mohammad R. Jalali ,et al ., (2007)[18], found that HER2/neu was positive in7.1% of grade I,28.6% of grade II and 65.4% of grade III ,P=0.002. Our findings state that there was no relationship between age group and HER-2\ neu overexpression in bladder carcinoma patients (P > 0.05). This is in agreement with Gokhan Ates , et al .,(2007)[17] and Mohammad R. Jalali ,et al .,(2007)[19]. Our results state that in TCC , there is male increasing significantly than female in relation to HER-2\neu protein overexoression (P < 0.05).

The immunohistochemical analysis of the results revealed that P53 overexpression was increasing as the grade increased, with significant difference among these grades (P = 0.014). However, it is significantly correlated to the grade of tumor (R = 0.821). Venyo A , et al .,(2012)[24] found that 28.88% of grade I ,66.66% of grade II and 80.7% of grade III were p53 positive, Salah A. Ali et al .,(2012)[20] show that The p53 expres-sion was increasing with increasing grade of tumor .There was statistically a highly significant correlation between p53 expression and grading of cases of TCC (p value < 0.001), Hong-Lin Cheng *et al.,* (2002) [25] showing that p53 immunostaining was positive in 26% of grade I, 57 % of grade II, and 42% of grade III. Findings of the current work also agreed by Nurcan Kilicli-Camur *et al.,*. (2002) [25], Ming-Lan Lu *et al.,* (2002) [22] , and Sunanda J *et al.,* (2004) [23]. While Underwood MA *et al.,* (1996) [26] found no predictive prognostic value for p53 immunohistochemistry over grade value and this may be due to the usage of polyclonal antibody to p53 in their study. In the presented study there was no relation between p53 expression and age p>0.05 In agreement with Salah A. Ali et al .,(2012)[20]. In our study, positive immunostaining of Her2 was reported in 38.88% in grade I transitional cell carcinoma, while P53 was positive in 44.44% of the same grade. In grade II Her2 was positive in 50% of cases compared with 60% positive P53 in the same grade. Finally Grade III showed 91.66% positive Her2 while P53 shoed 87.5%. Most of positive Herr bladder cancers were positive for P53. There was strong positive correlation between Her2/ neu and P53 regarding all the three grades. Yuh-Shyan T. et al.,(2005)[18] show similar correlations between HER2/neu with P53 overexpression in transitional cell carcinoma ,although Gunia S. ,et al .,(2011)[27] found a positive correlation between the two markers while evaluating their role in separating CIS from benign flat lesions of urinary bladder.

Conclusions

From the above results we concluded that p53 and Her_2/neu over-expression play an important role in pathogenesis of urothelial carcinoma evolution, as their positivity associated with higher tumor grade.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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