

**IMMUNOHISTOCHEMICAL EXPRESSION OF CD10 AND HER-2/NEU IN
UROTHELIAL CARCINOMA
VERSUS CHRONIC CYSTITIS IN ZAGAZIG UNIVERSITY
HOSPITALS(RETROSPECTIVE STUDY)**

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ABSTRACT

Background: Urinary bladder tumor is the seventh most common worldwide tumor. According to National Cancer Institute in Egypt, urinary bladder tumor constitutes 30% of all cancer cases with an incidence rate of 13.5/100,000 individuals. CD10 protein has been an immediate part in pathways of transduction that control cellular development, apoptosis, metastatic possibilities of cancer cells and influence on invasion. CD10 has prognostic incentive in bladder cancer. HER2/neu protein seems to assume a part in neoplastic cell and included in pathogenesis of urinary bladder carcinoma. Association of HER2 receptor in proliferation of invasive urothelial carcinomas has incited trials to HER2/neu targeted therapies in advanced diseases.

Aim of work: To evaluate the immunohistochemical expression of CD10 and HER2/neu with the pathological parameters of urothelial carcinoma of urinary bladder well as grade and stage versus chronic cystitis

Methods: CD10 and HER2/neu expressions were retrospectively analysed by immunohistochemistry in 30 cases of chronic cystitis and 30 paraffin-embedded specimens of patients with primary urothelial carcinoma (UC). The expressions were related to clinicopathological parameters, well as grade and pathological stage

Results: The immunohistochemical expression of CD10 and HER2/neu were negative (score 0) in chronic cystitis cases than urothelial carcinoma and was significantly ($p < 0.05$). Nineteen cases (63%) of primary urothelial carcinomas were positive for CD10 expression and 17 cases (56.7%) were positive for HER2/neu compared to chronic cystitis. The high grade of urothelial carcinoma and the high pathological stage were significantly connected with the high CD10 and HER2/neu expression ($p < 0.05$).

Conclusion: High CD10 and HER2/neu expression was related to high grade and pathological stage of urothelial carcinoma.

Keywords: CD10 – HER2/neu – urothelial carcinoma of urinary bladder – Immunohistochemistry (IHC)

INTRODUCTION

Urinary bladder tumor is the sixth most common tumor in the United States after lung tumor, prostate tumor, breast tumor, lymphoma and colon tumor. It is the third most common tumor in men and the eleventh most common tumor in women [1]. According to National Cancer Institute in Egypt, urinary bladder tumor constitutes 30% of all cancer cases with an incidence rate of 13.5/100,000 individuals. [2]. In spite of the major cases of bladder cancers, presented with tumor located in the superficial layer of the urinary bladder wall, approximately 20–40% of the cases will present with invasive tumor [3].

Urinary bladder cancer was morphologically heterogeneous; more than 90% of urinary bladder cancers was urothelial carcinoma (TCC), whereas primary squamous cell carcinoma (SCC), small cell carcinoma, adenocarcinoma and other tumors are less common [4]. The detection of new biological markers, may prompt to the enhancement of therapeutic approaches and clinical prediction of bladder cancer cases with the purpose of decreasing the danger of progression. [5]. The CD10 (common acute lymphoblastic leukemia antigen) was a surface zinc-dependent enzyme metalloprotease that played role in inactivation of different bioactive neuropeptides [6]. The CD10 is

/normally expressed in many tissues, including epithelial cells of the breast, kidney, intestine, prostate and lung; accordingly, disturbance of CD10 expression has been connected with development or progression in a variety of tumors [7]. Conflicting results have been accounted in bladder urothelial carcinoma, with CD10 down regulation involved in progressive tumors, or, inversely, it is up regulation has been connected with invasion and metastasis [8]. The HER-2/neu (human epidermal growth factor receptor 2) located by (c-erbB-2) gene that restricted to chromosome 17q21 and involved a transmembrane tyrosine kinase receptor protein [9].

As well as its value in controlling normal cellular proliferation, amplification of the HER2/neu gene appears to play a role in neoplastic cell growth [10].

Besides a role of HER2/neu in prognostic significance, assessment of Her-2/Neu expression in cases with urinary bladder cancer has been effected in therapeutic approaches [11].

This study aimed to assess immunohistochemical expression of HER2/neu and CD10 in chronic cystitis and urothelial carcinoma of urinary bladder and their relation to pathological parameters as, histopathological grade and stage.

MATERIALS AND METHODS

Patients and materials

The retrospective studied groups included random 30 cases of chronic cystitis (cystoscopic biopsies) and 30 cases of primary bladder UC selected from files archives of Pathology department, Faculty of Medicine, Zagazig University. Of these 17 cases were selected by TRUB procedures and 13 cases by radical cystectomies. Only TRUB cases with muscle fibers were included in the study.

Sections of hematoxylin and eosin were examined to confirm diagnosis. Grade of examined sections of urothelial carcinoma according to the classification of WHO 2016 [12] and were evaluated pathological stage (depth of invasion of urothelial carcinoma) according to TNM staging system tumors [13]. Independently of two experienced pathologists confirmed the histopathological

diagnosis of each sections and concurred on the grade and pathological stage of urothelial carcinoma. Immunohistochemically, other sections with positively-charge slides, stained with antibodies against HER2/neu and CD10.

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Immunohistochemically stained: the staining steps related to the instructions of manufacture. The recovery of antigen through microwave treatment in 10 mM of buffer citrate pH 6.0. Sections of lesions were been incubated with mouse monoclonal antibody against CD10 (Dako, U S A, Cat, M S 56C6, getting ready to use) & mouse monoclonal HER2/neu (Lab Vision, U S A, Cat. MA1-34495, getting ready to use). The slides were been incubated for (60 minutes) with each antibody at room temperature. The DAB, chromogen substrate solution was been applied.

Positive control For CD10, tonsil sections were used. A positive control for HER2/neu was section of invasive ductal breast carcinoma positive for HER2/neu. Negative controls were set up by excluding primary antibody.

Immunohistochemical evaluation

Scoring of immunohistochemical expression of CD10:

The cytoplasm and or cell membrane staining was regarded as positive expression. A semiquantitative score depended on the percentage (%) of positive cells of the tumor was assessed with respect to this score, Score 0: Means that (< 5%) of cells showed membrane and or cytoplasmic staining (negative), Score 1: Means that (5-50%) of cells showed membrane and/or cytoplasmic staining (positive) and Score 2: Means that (>50%) of cells showed membrane and or cytoplasmic staining (positive) [14].

Scoring of immunohistochemical expression of HER2/neu: sections of immunostaining were assessed under light microscope independently by two pathologists. Only membranous staining was regarded as positive with respect to this scoring: Score 0: Mean that no staining or membrane staining showed in < 10% of the tumor. Score 1: Mean that Faint partial membrane staining showed in >10% of the tumor cells. Score +2: Mean that Weak to

moderate circumferential staining showed in > 10% of the tumor cells .Score +3 : Strong circumferential membrane staining showed in > 10% of the tumor cells [9].

Statistical analysis:

The study was analyzed statistically by the SPSS (version 16.0 of windows) software package regarding to Spearman's correlation coefficient. Correlation between different variables were computed by Fisher's exact test. p-value < 0.05 was regarded as significant (S) and p value > 0.05 was regarded as non statistically significant (N S).

RESULTS

Regarding to sex distribution among the studied cases, males represented 34 cases which included 10 cases chronic cystitis ,24 cases urothelial carcinoma while females represented 26 cases which included 20 cases chronic cystitis , 6 cases of urothelial carcinoma. The studied cases of chronic cystitis with mean age of 40 years while the main age of urothelial carcinoma is 64 years . The low grade were 12 cases(i.e. grade I,II) and the high grade(i.e. grade III,IV) were 18 cases. According to pathological stage of urothelial carcinoma were classified into PTa showed 7 cases , stage PT1 showed 5 cases, PT2 stage showed 8 cases and PT3 stage showed 10 cases . No cases were T4 reported

A- Immunohistochemical results of CD10 staining: In chronic cystitis cases, all cases are negative for CD10 (Fig 1) . In urothelial carcinoma , 19 cases (63%) were positive immunostaining . Statistically significant relation was presented between CD10 expression and examined lesions (p <0.05)(Table1).

The statistically significant relation was presented between CD10 and grading of UC (p < 0.05) and pathologic stage (p < 0.05) (Table 1). Expression of CD10 was increased in high grading of UC and high stage of UC (Fig. 2)

B- Immunohistochemical results of HER2/neu staining:

All chronic cystitis cases were showed negative score of HER2/neu expression (Fig 1), while 17 cases of UC (56.7%) were a positive staining with statistically significant relation (p < 0.05) { Table 2}.

A statistically significant relation between expression of HER2/neu and grading of UC (p < 0.05). High grade of UC were associated with higher HER2/neu expression. A significant statistical relation was observed between expression of HER2/neu and pathologic stage (p < 0.05) {Table 2} (Fig. 3).

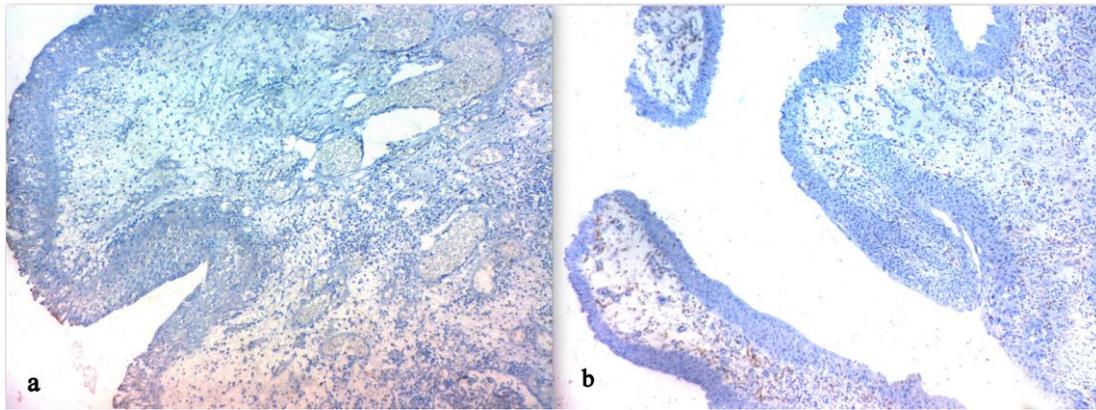
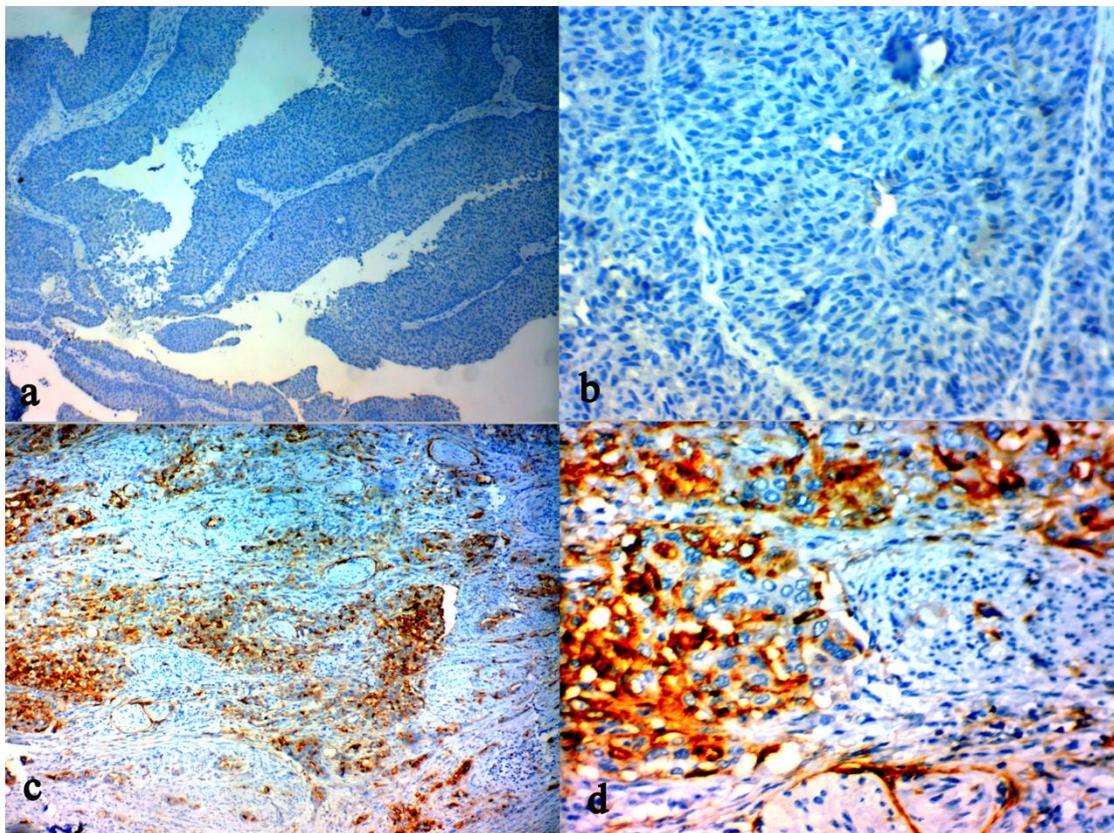
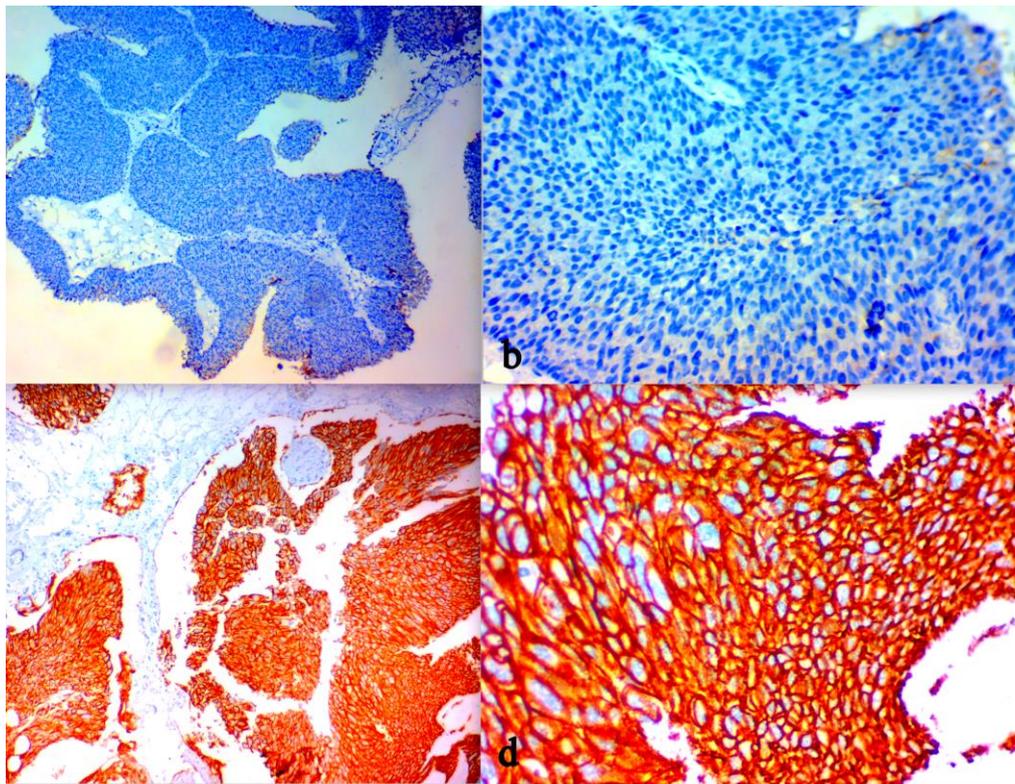


Figure (1): Polypoid cystitis .(a): Negative HER2/neu immunostaining (score 0) ,(x400).(b): Negative CD10 immunostaining (score 0),(x 200)



figure(2): immunohistochemical CD10 expression a) Papillary urothelial carcinoma (PTa) showed negative immunostaining (CD10 score 0), (x200). b) Low grade papillary urothelial carcinoma showed negative immunostaining (CD10 score 0) (x400).c) Invasive urothelial carcinoma (PT2) with positive immunostaining (CD10 score 2) (x200).d) Previous case showed high grade invasive urothelial carcinoma with strong positive immunostaining (CD10 score 2), (x400)



Figure(3): Immunohistochemical HER2/neu expression, a) papillary urothelial carcinoma (PTa) showed negative immunostaining (score 0), (x200). b) Previous case showed low grade papillary urothelial carcinoma negative immunostaining (score 0), (x400). c) Invasive urothelial carcinoma (PT3) showed strong positive(HER2/neu score3), (x200)d) Previous case high grade urothelial carcinoma showed strong positive (Her2/neu score 3)

Table(1):Correlation between CD10 expression and clinicopathological data of examined cases:

Characteristics	N	CD10 expression		χ^2	p-value
		Negative (0 score)	Positive (1&2 score)		
Type of tissue examined:					
Chronic cystitis	30	30 (100%)	0 (0%)	0.5	< 0.001 (S)
Urothelial carcinoma	30	11 (36.7%)	19 (63.3%)		
Sex of tumor					
≤ 60y	14	7 (50%)	7 (50%)	0.6	0.3 (NS)
>60y	16	7 (43.8%)	9 (56.2%)		
Gender of tumor					
Male	24	10 (41.7%)	14 (58.3%)	0.7	0.4 (NS)
Female	6	1 (16.7%)	5 (83.3%)		
Tumor grade					
Low grade	12	8 (66.7%)	4 (33.3%)	3.8	0.008 (S)
High grade	18	5 (27.8)	13 (72.2%)		
Pathological stage (T)					
PTa	7	4 (57.1%)	3 (42.9%)	6.3	0.03 (S)
PT1	5	2 (40%)	3 (60%)		
PT2	8	3 (37.5%)	5 (62.5%)		
PT3	10	4 (40%)	6 (60%)		

χ^2 : Chi-square test.; NS: Non significant.; S: significant; p< 0.05 is significant.

Table(2): Correlation between Her2/neu expression and clinicopathological data of examined cases:

Characteristics	N	HER2/neu expression		χ^2	p-value
		Negative (0&1 score)	Positive (2&3 score)		
Type of tissue examined:					
Chronic cystitis	30	30 (100%)	0 (0%)	0.5	<0.001 (S)
Urothelial carcinoma	30	13 (40.3%)	17 (56.7%)		
Sex of tumor					
≤ 60y	14	7(50%)	7 (50%)	0.2	0.8 (NS)
>60y	16	9 (56.2%)	7 (43.8%)		
Gender of tumor					
Male	24	10 (41.7%)	14 (58.3%)	1.7	0.2 (NS)
Female	6	1 (16.7%)	5 (83.3.9%)		
Tumor grade					
Low grade	12	8 (66.7%)	4 (33.3%)	4.4	0.03 (S)
High grade	18	3 16.7(0%)	15 (83.3%)		
Pathological stage (T)					
PTa	7	5 (71.4%)	2 (28.6%)	3.9	0.008 (S)
PT1	5	3 (60%)	2 (40%)		
PT2	8	1 (12.5%)	7 (87.5%)		
PT3	10	2 (20%)	8 (80%)		

χ^2 : Chi-square test.; NS: Non significant; S: significant; p< 0.05 is significant.

DISCUSSION

Urinary bladder tumor have different molecular alteration and difficult biological pathways which control cellular development, proliferation, apoptosis, differentiation, angiogenesis and metastasis. CD10 molecule participate in the control of cell growth, differentiation, adhesion, angiogenesis and invasion by enzymatic hydrolysis of different peptide hormones. These processes are important in normal physiology, in addition to development and progression of tumor [7].

In current study, expression of CD10 was detected in urothelial carcinoma in 63.3% (19 out of 30 cases) and completely absent in chronic cystitis. In agreement with Kandemir et al [7] and Atique et al. [14] showed higher expression of CD10 was reported in urothelial carcinoma compared to non-neoplastic urothelium. In contrast with Murali et al. [15] detected expression of CD10 in 50% of non neoplastic urothelium samples and in 67% of urothelial carcinoma that his study take large samples of non-neoplastic lesions while Bircan et al. [16] were found CD10 expression in 9.9% of non neoplastic urothelium and in 43% of urothelial carcinoma.

As regard to our results, expression of CD10 in relation to grade of urothelial carcinoma, a positive rate of CD10 immunoreactivity was detected in 33.3% (4 out of 12 cases) of low grade tumor and 83.3%(15 out of 18 cases) in high grade of UC. CD10 expression showed statically significance relation with urothelial carcinoma grade (p=0.01), similar results were obtained by Murali et al. [15]; Bahadir et al. [17]; Kandemir et al. [7]; Mohammed et al. [6] and Atique et al. [14] and disagreed with other studies were done by Koiso et al. [18] and Bircan et al. [16] Whom take large sample size for used

As regard to our results, analyzed CD10 expression in relation to pathological stage (T) in urothelial carcinoma, CD10 expression was detected in 28.6% of PTa of tumors (2 out of 7 cases), 60% of PT1 of tumors (3 out of 5 cases), 75% of PT2 (6 out of 8 cases) and 80% of PT3 of tumors(8 out of 10 cases). There was satatically significance relation between CD10 expression and pathological stage of urothelial carcinoma (depth of invasion of tumors) which was in agreement with a studies done by Abdou [19] and Bahadir et al. [17] while disagreed with other study done by Kandemir et al. [7]. who found no significant correlation between immunohistochemical expression of CD10

and the pathological T-staging of urothelial carcinoma. Such discrepancies can be explained by difference in sample size, moreover, improper staging of the specimens due to subjective errors in assessing the stage or improper transurethral resection technique in which deeper tissues especially the muscular layer had not been taken, may contribute to under or over estimation of staging in these cases which in turn affects the number of cases in each stage. CD10 immunohistochemical marker had used a different scoring method such variations in scoring systems will definitely affect the number of positive cases and affecting the correlation significance .

Many authors were assessed HER2/neu overexpression in urothelial carcinomas , because the HER2/neu protein is implicated in pathogenesis of urothelial carcinoma, as important as in breast cancer ^[21]

The frequency of HER2/neu overexpression in urothelial carcinoma ranges between 12% ^[21] and 71% ^[22] , these variations in reported rates of Her2/neu expression are due to differences in applied scoring criteria for the assessment of the HER2/neu overexpression or could to tumor heterogeneity , collecting samples and method used

In the current study was used immunohistochemical method for assessment of HER2/neu expression, which was the most common method intended for breast tumor . This study showed positive expression of HER2/neu in 56.7% of urothelial carcinomas and this rate were within previously series of published data; Caner et al., ^[23] ; Naik et al., ^[20] and El Gehani et al., ^[22] confirming the effective of immunohistochemical method in the evaluation of HER2/neu expression in urothelial carcinoma .In agreement with Al-Tereihi et al. ^[24] and Hammam et al. ^[25] , all cases of chronic cystitis showed negative score of HER2/neu expression, whilst 56.7% cases of urothelial carcinoma showed positive scoring . The expression of HER2/neu was not identified in chronic cystitis cases so inflammatory urothelium seemed not to affect on HER2/neu expression. In comparison with Hammam et al. ^[26] that HER2/neu expressed in bilharzial

cystitis because their study take a large sample of bilharzial cystitis, with significant difference ($p < 0.05$) . Overexpression of HER2/neu showed different rank in this study by reason of small sample size. In this study showed variance in overexpression of HER2/neu of high grading UC in relative to low grading UC with statistically significant ($p < 0.05$), the high grade of urothelial carcinoma showed high overexpression than low grade showed lower expression so the HER2/neu expression was statistically significantly related to the differentiation of tumors . This study was found a significant relationship between expression of HER2/neu score and pathological stage of urothelial carcinoma ($P < 0.05$). In agreement with several reports ^[21-27-28-29] whom found that overexpression of HER2 /neu protein correlated with the grade and stage of the urothelial carcinoma . In comparison with Ioachim et al. ^[30] who found no relation between her2/neu over expression and the grade and stage of UC ($P > 0.05$). This difference results due to the heterogeneity between antibodies, protocols, interpretations of HER2/neu expression .

In conclusions: Using immunohistochemical method , high expression of CD10 was related to high grade and stage of urothelial carcinoma . HER 2/ neu overexpression was associated with high grade and stage of urothelial carcinoma compared with low grade and stage of UC.

Comprehensive research and the large sample sized can detected the credible value of HER2/ neu and CD10 expression in pathogenesis of UC and its prognostic factors in bladder urothelial carcinoma .

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