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ORIGINAL PAPER

HER2/neu expression status of post BCG recurrent non-muscle-invasive bladder urothelial carcinomas in relation to their primary ones

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Summary ***Background: Transurethral resection (TUR) followed by adjuvant therapy is still the treatment of choice of Non-Muscle-Invasive Bladder Urothelial Carcinoma (NMIBUC). However, recurrence is one of the most troublesome features of these lesions. Early second resection and adjuvant BCG therapy has been shown to improve the outcome.***
Objective: To evaluate the prognostic value of C-erbB-2 (HER2/neu) expression status in Non-Muscle-Invasive Bladder Urothelial Carcinoma cases, before and after intravesical Bacillus Calmette Guerin (BCG immunotherapy).
Materials and methods: HER2/neu expression was studied in 120 (Ta-T1) Non-Muscle-Invasive Urothelial Carcinoma cases. The expression was evaluated and compared to the expression after Bacillus Calmette Guerin (BCG) immunotherapy.
Results: HER2/neu expression in low and high grade of the Non-Muscle-Invasive Urothelial Carcinoma was (38%) and (83%) respectively. The difference of the expression rates by tumor grade was statistically significant. In recurring lesions post BCG therapy, C-erbB-2 expression was markedly decreased (31.6%) when compared to its expression before therapy (65%).
Conclusions: The HER2/neu expression increased as the tumor grade rose. The reduction in expression following BCG treatment in Non-Invasive transitional cell carcinoma cases could reflect a reduction of the potential malignancy of the tumor.

KEY WORDS: Transurethral resection; Non-Muscle-Invasive Urothelial Carcinoma; C-erbB-2; Bacillus Calmette Guerin; Immunohistochemistry.

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INTRODUCTION

Non-Muscle-Invasive Bladder Urothelial Carcinoma (NMIBUC) is the most common type of urinary bladder carcinoma. It accounts for approximately 75% of all BC lesions at initial presentation; this percentage is even greater in younger patients (< 40). The high prevalence of NMIBUC can be attributed to its long-term survival and reduced risk of cancer-specific mortality compared to muscle-invasive (T2-4 stages) tumors (1). These tumors are restricted to the mucosa (Ta, CIS) or submucosa (T1), and are treated by trans-urethral resection. Although *trans-urethral resection of bladder tumors* (TURBT) by itself can totally remove Ta/T1 lesions, they frequently recur and can progress to MIBC. The recurrence rate of such tumors is 50 to 70%. As a result, all patients should be considered for adjuvant *intravesical instillation* (IVI) therapy and surveillance, based on their risk stratification (2). The most important step in the management of NMIBUC is the transurethral resection procedure. This procedure is crucial for the complete removal of all visible/suspicious lesions and for proper grading and staging by sampling of detrusor muscle; thus, determining the next appropriate treatment (2). Because of the high risk of recurrence and progression following primary resection, adjuvant therapy and long-term surveillance should be considered in all patients (3). There is evidence that treatment with *Bacillus Calmette-Guérin* immunotherapy following primary resection can lower cancer recurrence rates and progression to more advanced stages (4).

C-erbB-2 is a tyrosine kinase transmembrane protein that is related to *epidermal growth factor receptor* (EGFR) family and it is known as HER2/neu (human epidermal growth factor receptor-2). Its expression in urinary bladder transitional cell carcinoma has been described, and it has been proposed that its expression increases with tumor grade and recurrence of urothelial cancer. Bladder urothelial carcinomas with C-erbB-2 expression have poor prognosis, hence

it can be used as a prognostic clinical biomarker (5). The goal of this study is to evaluate the C-erbB-2 (HER2/neu) immunostaining status in Non-Muscle-Invasive Bladder Urothelial Carcinoma cases, before and after intravesical *Bacillus Calmette Guerin* (BCG) immunotherapy.

Patients and methods

This work was conducted at our institution over the period between March 2016 to February 2020. It included 120 cases (86 men and 34 women; mean age 52.3 ± 11.4, range 41-70 years) with recurrent urothelial tumors confined to the bladder. All patients had a history of *transurethral resection of bladder tumor* (TURBT) and full dose BCG therapy for primary NMIBUC lesions. Before removal of the tumor, each patient had a history taken, physical examination, urinalysis, an *ultrasound scan* (USS), and CT scan. TURBT was performed under classical white light using the 26-Fr. continuous-flow Karl Storz resectoscope with distilled water for irrigation. The aim of resection was complete removal of all visible/suspicious lesions and their underlying muscularis propria. The tumor and its related underlying muscle were sent separately labelled for histopathologic assessment. Tumors were staged and graded according to the WHO/ISUP classification 2016 with external consultation when indicated. We selected patients who had NMIBUC (Ta-T1) without *carcinoma in situ* (Cis) after pathological examination. A second look cystoscopy was then performed after 2 weeks of the initial resection to ensure no residual lesion. Patients were then followed up for *intravesical instillation* (IVI) of BCG. BCG was instilled for six consecutive weeks, as an induction dose, starting 15-21 days after TURBT to enable surface urothelium to recover (6). Two weeks after the last instillation, surveillance cystoscopy was carried out, with random cold-cup biopsies taken from the ex-tumor site and its vicinity, as well as from other regions of the bladder, to detect recurrence. The emergence of histopathologically confirmed urothelial tumor in the bladder, regardless of stage, was characterized as recurrence. Since the biopsies were negative, our patients received a maintenance dose of BCG (3-weekly instillations, given at 3, 6, 12, 18, 24, 30 and 36 months).

Histopathology

Prepared H&E slides were analyzed and examined microscopically by the histopathologists to confirm the diagnosis according to the diagnostic criteria defined by the latest WHO/ISUP classification 2016.

Immunohistochemistry

HER2/neu Immunostaining

An automatic immunohistochemical staining device (*Benchmark XT; Ventana Medical System, Tucson, Arizona, USA*) was used to stain formalin-fixed paraffin-embedded tissue sections, according to the manufacturer's instructions.

Briefly, 5-mm-thick sections were cut on Poly-L-lysine-coated adhesive slides and dried for 30 minutes at 62°C. After epitope retrieval by standard heat treatment for 30 minutes in ethylene diamine tetra acetic acid (pH 8.0) in an autostainer, the samples were incubated with mouse polyclonal antibodies to C-erbB-2 (*dilution1: 500, cloneA0485; Labvision/Neomarker, Fremont, California, USA*). The slices were then counterstained with Harris hematoxylin after being treated with biotinylated anti mouse immunoglobulins, peroxidase-labeled streptavidin (*LSAB Kit; Labvision*), and 3.30-diaminobenzidine. Breast cancer slices fixed in paraffin were used as positive controls. Membrane staining was assessed. This step was evaluated independently and jointly by the histopathologists.

HER2/neu expression

The percentage of stained cells and intensity of staining were ranged from 0 to 3+, as follows: no staining (0), low intensity and incomplete membrane staining in less than 10% of cells (1+), low intensity and full membrane staining in more than 10% of cells (2+), and high intensity and total membrane staining in more than 10% of cells (3+). Tumors with scores 0 and 1+ were considered to be negative, while those with scores 2+ and 3+ were considered positive (7).

Statistical analysis

The collected data were arranged, tabulated, and statistically analyzed using SPSS software statistical computer package version 16 (*SPSS Inc., Chicago, Illinois, USA*).

Student's t-test was used to evaluate the difference between two means. The Mann-Whiney test was used to compare two different groups, while the Wilcoxon signed rank test was used to compare two related groups. The threshold for significance was set at less than 0.05.

Results

The 120 included patients had NMIBUC lesions, with a tumor size of 8-20 mm. The lesions were detected by USS in 58 patients and by CT in the remaining 62 patients.

Immunohistochemical findings

C-erbB-2 expression was found in 77 of 120 primary NMIBUC cases (64.16%) (Table 1) with expression ranging from weak (score 2 +) in 29 cases to strong (score 3 +) in 48 cases (Figures 1 and 2). When the score and tumor grade were compared, it was observed that C-erbB-2 was expressed in 19 out of 50 in low-grade cases (38%).

In high grade cases, C-erbB-2 was expressed in 58 out of 70 instances (83%), with expression ranging from weak (score 2 +) in 19 cases to strong expression (score 3 +) in 39 cases. C-erbB-2 expression in high grade urothelial

Table 1.
C-erbB-2 expression in of the primary NMIBC lesions.

Groups		No	C-erbB-2 expression					
			-VE	+VE			NO (%)	Total/NO (%)
			0	+1	+2	+3		
Non-invasive urothelial carcinoma	Low grade	50	21	10	10	9	19/50 (38%)	77/120 (64.2%)
	High grade	70	12	0	19	39	58/70 (83%)	
P value								< 0.05

Mann-Whitney test used. BCG, Bacillus Calmette Guerin.

Figure 1.

Non-Muscle-Invasive Bladder Urothelial Carcinoma (NMIBUC) limited to lamina propria - (Low grade T1) (a) H&E X200, (b) Positive (score +2) for C-erbB-2 (membranous) - 200, (c) The same cases after B.C.G therapy negative for C-erbB-2 (score zero) x 200.

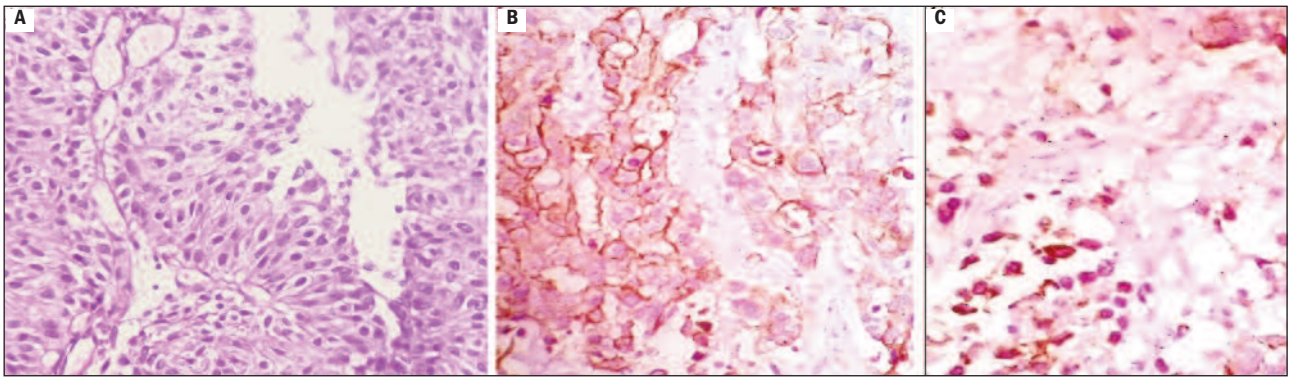
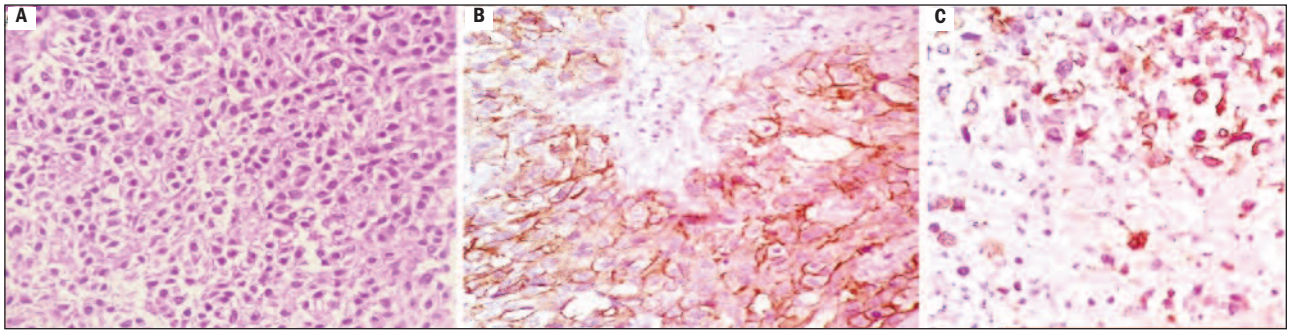


Figure 2.

Non-Muscle-Invasive Bladder Urothelial Carcinoma (NMIBUC) limited to lamina propria - (High grade T1) (a) H&E X200, (b) Strong positive (score + 3) for C-erbB-2 (membranous) - x 100, (c) The same cases after B.C.G therapy negative for C-erbB-2 (score 1) x 100.



carcinoma was significantly higher when compared to low grade one ($p < 0.05$). Hence, with increasing tumor grade, there was a statistically significant rise in C-erbB-2 expression.

C-erbB-2 was found in 38 out of 120 cases (31.6%) of recurring lesions post BCG therapy with expression ranging from weak (score 2 +) in 28 cases to strong (score 3 +) in 10 cases. Interestingly, its expression was found only in 8 only out of 50 low grade (16%), and in 30 out of 70 high grade (42.9%) recurring lesions. This drop in C-erbB-2 expression in recurring cases after BCG therapy was statistically significant ($p < 0.005$) when compared to its expression in the same group before therapy.

Discussion

Histologically, 90% of bladder cancer lesions are of urothelial origin. At the time of diagnosis, 20-25% of these lesions are Muscle-Invasive (stageT2 or higher); the rest are Non-Muscle-Invasive, previously called superficial bladder cancer. Only (8-12%) of all NMIBC tumors may progress to *Muscle-Invasive Bladder Urothelial Carcinoma* (MIBUC) (8).

The commonly employed scoring systems for initial risk stratification of NMIBUC lesions and for prediction of their risk of recurrence and progression after IVI of BCG, are based on clinicopathologic factors: age, gender, tumor (number, diameter, grade, and staging), prior recurrence status, and concurrent CIS (9). Aside from these clinicpathologic criteria, it would be advantageous if biological markers could contribute in the risk categorization of NMIBUC lesions and in predicting their risk of recurrence and progression after IVI therapy. Molecular biomarkers such as FGFR3, p53, p63 and Epidermal Growth Factors, and their prognostic role have been investigated in many studies (10, 11, 12). According to the latest EAU guidelines, the role of these biomarkers in the current era of personalized cancer management is promising, especially in the patients' categorization based on molecular classification (2). Regrettably, existing evidence is still inconclusive, and these biomarkers are not yet ready for routine use in clinical practice (13). C-erbB-2 is a tyrosine kinase receptor that belongs to the epidermal growth factor receptor family. It regulates the cell cycle and promotes cell growth. C-erbB-2-positivity rates have been found to vary between populations in studies. The majority of C-erbB-2 expression investigations in bladder cancer have been conducted on MIBC, with expression ranging from 9% to 81% (14).

Another tyrosine kinase dysregulation of Axl receptor and its ligand growth arrest specific gene was also studied in urinary bladder carcinoma and showed a close relation to tumor stage and tumor grade, but still further

studies are recommended to assess its role in tumor prognosis (15, 16).

However, there are few reports of C-erbB-2 status in NMIBUC. C-erbB-2 protein overexpression has been found in 4-13% of NMIBUCs in a few studies (12, 17).

In the current study, we investigated the immunohistochemistry expression of C-erbB-2 in 120 patients with recurrent NMIBUC, comparing it with the expression of their primary lesions. We found that C-erbB-2 expression was seen in 64.16% (77 cases) of the primary tumors and there was a significant relationship between C-erbB-2 expression, in both recurrent and initial lesions, with tumor grade. There is a statistically significant increase in C-erbB-2 expression as tumor grade rises. These results were in line with those of *Hegazy et al.* in 2015 and *Agrawal et al.* in 2020 (10, 12), who found a relation between greater tumor grade and increased C-erbB-2 expression. BCG immunotherapy post TURBT remains the most effective treatment for reducing the risk of NMIBUC recurrence and progression. Patients with recurrent NMIBUC (with or without Cis) are frequently administered adjuvant intravesical BCG (18). According to *Hegazya et al.* and *Morgan et al.* (10, 19), BCG adjuvant therapy for NMIBUC lowered the incidence of C-erbB-2 expression with favorable outcomes. In our research, we observed a significant drop in C-erbB-2 expression in recurrent lesions (38/120) when compared to their primary ones (77/120) ($p < 0.05$). Overall, an accurate assessment of C-erbB-2 status is recommended in NMIBUC lesions for proper patient selection to BCG therapy. In primary lesions, the C-erbB-2 immunoreactivity should be assessed before initiation of BCG immunotherapy. If there is recurrence, the C-erbB-2 expression of the recurring lesion should be performed and compared with the primary one. In case of down expression, one can consider adjuvant BCG therapy.

Future studies, particularly ongoing C-erbB-2 targeted therapy trials, will undoubtedly shed further light on the significance of C-erbB-2 in bladder cancer management and treatment. However, BCG production has shown its limits, with recent worldwide BCG shortage (20).

Conclusions

This study contributes to our understanding of C-erbB-2 expression in urothelial carcinoma. It supports the role of C-erbB-2 immunostaining in NMIBUC lesions since the expression increased with increase tumor grade. Our findings also suggest the importance of BCG therapy in the treatment of these cases although further studies on the role of this marker in recurrent lesions are highly recommended.

Ethical Approval: Provided from *Damietta Faculty of Medicine* Under Id number: DFM-IRB-00012367 - 23-03-003.

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