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# The clinical significance of HER2 protein amplification/expression in urinary bladder lesion

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**KEYWORDS**

HER2;
Bladder cancer;
Transitional;
Squamous;
Schistosomiasis

**ABBREVIATIONS**

IHC, immunohistochemical;
FISH, fluorescence *in situ* hybridisation;
SCC, squamous cell carcinoma;

**Abstract** ***Objective:*** To evaluate HER2 oncoprotein expression by both immunohistochemical (IHC) staining and fluorescence *in situ* hybridisation (FISH) in different benign and malignant bladder lesions, and the effect of bilharzial infestation on this expression.

***Patients and methods:*** In a prospective controlled study, 72 patients were classified into a control group, and groups with cystitis, urothelial carcinoma, and squamous cell carcinoma (SCC). HER2 was detected using standard IHC staining and FISH in all groups. The correlation of HER2 expression with tumour type, stage and grade in relation to normal urothelium and cystitis was assessed. The effect of schistosomal infestation was evaluated.

***Results:*** HER2 expression was statistically significantly higher in patients with malignant lesions than in the other groups, and in high-stage and -grade tumours than in low-stage and -grade tumours. The use of FISH increased the detection of HER2-positive tumours. Schistosomal infestation did not affect HER2 expression in patients with transitional cell carcinoma.

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TURBT, transurethral resection of bladder tumour;
(N)MI, (non-)muscle-invasive;
EGFR, epidermal growth factor receptor

***Conclusion:*** High-stage and -grade bladder malignancies expressed HER2 much more than did benign lesions. FISH is more sensitive for detecting HER2 expression. The treatment of HER2-positive tumours might benefit from novel targeted-treatment protocols.



## Introduction

Carcinoma of the bladder is a world-wide health problem, and ranks ninth in cancer incidence rates [1]. The highest incidence rate of bladder cancer is recorded in Egypt (37.1 per 100,000 males) [2]. Analysis of pooled data showed bladder cancer to comprise 30.3% of all cancers, with a male to female ratio of ≈3:1 [3].

The standard treatment for muscle-invasive (MI) urothelial bladder cancer is radical cystectomy, which is also the final treatment for refractory non-MI (NMI) TCC of the bladder, together with other histological types, including squamous cell carcinoma (SCC), which accounts for ≈40% of bladder cancers in Egypt [3].

The recurrence rate after cystectomy approaches 50%, indicating that this treatment alone might not be optimal for some patients [4]. Targeted therapy is a novel treatment strategy in oncology, targeting specific signalling pathways within the malignant cells, and that might enhance the cytotoxic effect but with fewer adverse events [5].

The HER2 gene contributes to physiological mechanisms of cell proliferation by intrinsic tyrosine-kinase activity. It is also termed the NEU, EGFR2, or ERBB2 gene, and is one of the members of the epidermal growth factor receptor (EGFR) family, which includes EGFR (or ERBB1), EGFR3 (or HER3/ERBB3) and EGFR4 (or HER4/ERBB4). The overexpression of HER2 was shown in several malignancies, and it is known to affect proliferation, angiogenesis and metastasis of malignant cells [6].

Overexpression of HER2 has been associated with different types of malignancies [7]; it was over-expressed in up to 34% of invasive breast cancers [8], gastric and colonic carcinomas [9,10], and bladder cancer [11]. In bladder cancer HER2 was reported to have one of the highest rates of expression, with up to 34% of the tumours being HER2-positive [12].

Trastuzumab is a monoclonal antibody which specifically targets HER2 protein by directly binding to an extracelluar protein receptor. Trastuzumab is used to treat both primary and metastatic breast cancer, and was shown to improve survival rates [13]. This has led to an evaluation of its efficacy and anti-tumour activity in patients with HER2 expression in bladder cancers.

Analysing gene amplification and/or increased protein expression of HER2 by various methods has produced different results [14]. Most of this controversy appears to have been caused by technical variables associated with the different methods used for detecting HER2 changes, especially the difference between gene amplification and overexpression [15].

Thus in the present study we evaluated the expression of HER2 protein using both immunohistochemical (IHC) staining and gene amplification by fluorescent *in situ* hybridisation (FISH) in different inflammatory and neoplastic urinary bladder lesions, including both TCC and SCC of the bladder. The effect of schistosomal infestation on HER2 expression in these lesions was also assessed and compared with normal urothelium as a control.

## Patients and methods

This prospective unrandomised study was approved by the institutional review board, and included patients with radiologically diagnosed bladder lesions listed for transurethral resection of bladder tumour (TURBT) in our Department, together with patients listed for cystoscopy for persistent LUTS. Patients with a previous history of bladder neoplasia were excluded. Ten patients who were examined by cystoscopy as a part of their urological evaluation and/or treatment served as a control. The final study included 72 patients (49 men and 23 women, median age 53 years, range 30–79). Patients were divided into the control group (10), a group with cystitis (either schistosomal-associated or nonspecific cystitis, 10), a group with SCC (19), and a group with TCC (MI and NMI, 33) (Table 1). All patients with an apparent tumour had TURBT, and cold-cup biopsies were taken from bladder mucosa of patients with LUTS and those in the control group.

Specimens were fixed in 10% buffered formalin, paraffin-embedded and processed routinely. Haematoxylin and eosin stains were used to evaluate all bladder lesions and to assess the grade and stage of carcinomas according to the international histological classification of urinary bladder tumours proposed by the WHO in 2004.

The expression of HER2 was evaluated using light microscopy at ×400 magnification, and the percentage of the positively stained cells was calculated and scored according to Gårdmark et al. [16], as 'negative' (score 0 or 1, where 0 is no staining or membrane staining in

**Table 1** The clinico-pathological distribution in the various groups, and the HER2 protein and gene immunoexpression by IHC and HER2 gene expression using FISH.

| Group | *N* | M/F | HER2 by IHC (score), *n* or *n* (%) | | | HER2 by FISH, *n* or *n* (%) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Negative (0/1+) | Equivocal (2+) | Strongly positive (3+) | Not amplified | Polysomy | Amplified |
| Control | 10 | 8/2 | 10 | 0 | 0 | 10 | 0 | 0 |
| Cystitis | 10 | 6/4 | | | | | | |
| Bilharzial | 6 | | 6 | 0 | 0 | 6 | 0 | 0 |
| Not bilharzial | 4 | | 4 | 0 | 0 | 4 | 0 | 0 |
| SCC | | | | | | | | |
| Bilharzial | 19 | 12/7 | 9 (47) | 3 (16) | 7 (37) | 7 (37) | 2 (11) | 10 (53)[a] |
| Not bilharzial | 0 | | | | | | | |
| TCC | 33 | 23/10 | 13 (39) | 11 (33) | 9 (27) | 20 (61) | 2 (6) | 11 (33)[c] |
| Ta | 13 | | 10 | 3 | 0 | 12 | 0 | 1[b] |
| T1 | 7 | | 0 | 4 | 3 | 3[d] | 1 | 3 |
| T2–3 | 13 | | 3 | 4 | 6 | 5 | 1 | 7[e] |
| Low-grade | 9 | | 6 | 3 | 0 | 8 | 1 | 0 |
| High-grade | 24 | | 7 (29) | 8 (33) | 9 (38) | 12 (50) | 1 (4) | 11 (46)[f] |
| Bilharzial | 16 | | 9 (56) | 2 (13) | 5 (31) | 10 (63) | 1 (6) | 5 (31) |
| Not bilharzial | 17 | | 4 (24) | 9 (53) | 4 (24) | 10 (63) | 1 (6) | 6 (35)[g] |

FISH vs. IHC.
[a] $P < 0.01$, positive HER2.
[b] $P < 0.05$, positive HER2.
[c] $P < 0.01$, positive HER2.
[d] $P < 0.1$, negative HER2.
[e] $P < 0.01$, positive HER2.
[f] $P < 0.01$, positive HER2.
[g] $P < 0.01$, positive HER2.

< 10% of tumour cells, and 1 is faint/barely perceptible membrane staining in > 10% of tumour cells, with cells only stained in part of their membrane), 'equivocal' (score 2, weak to moderate complete membrane staining in > 10% of tumour cells) or 'strongly positive' (score 3, strong complete cell membrane staining in > 10% of the tumour cells).

FISH analysis was used on a representative proportion of the tissue, using the Path Vysion kit (Abbott Laboratories. Abbott Park, IL, USA). All samples with positive HER2 protein expression were evaluated using labelled probes for both fluorophores, i.e., Vysis CEP 17 17p11.1-q11.1 Alpha Satellite DNA Spectrum Green, and Vysis LSI HER-2/neu 17q11.2-12 Spectrum Orange.

Slides were viewed with a fluorescence microscope (BX51 upright, Olympus Corp, Japan), where three areas were identified and in each area 20 nuclei were assessed. Chromosome 17 copy number and HER2 copy number were assessed for each of the 20 nuclei at ×1000 magnification. A ratio of chromosome 17 copy number over HER2/neu copy number was obtained from the 60 nuclei.

HER2/neu was classified based on the value used in breast cancer diagnostics into 'amplified' (Her2/neu/centromere 17 ratio > 2.2) 'not amplified' (Her2/neu/centromere 17 ratio < 2) or polysomy (more than one chromosome within the nucleus) [17].

Standard statistics were used for data management and analysis, assessing the relationship between variables using Spearman's correlation coefficient. All tests were two-tailed and considered statistically significant at $P < 0.05$.

## Results

### *Immunohistochemical studies of HER2 protein*

HER2 protein was not expressed in both the control and the cystitis group (Fig. 1A and B), while there was strong expression in 31% (16/52) malignant cases ($P < 0.01$). Schistosomal infestation appeared not to affect the HER2 expression in patients with cystitis.

There was HER2-positive expression in 27% of patients with TCC (Fig. 1C and D), while 37% of patients with SCC were positive for HER2 ($P < 0.01$; Fig. 1E and F).

Schistosomal infestation in patients with TCC was associated with a statistically significantly higher expression of HER2 than in non-schistosomal TCC ($P < 0.01$), and all cases of SCC were associated with schistosomal infestation (Table 1).

On stratifying TCC according to degree of muscle invasion, HER 2 was equivocally expressed in 23% of Ta tumours, and positively expressed in 43% and 46% of T1 and T2–3 tumours, respectively, with a statistically significant increase in the percentage of HER2 protein immunoexpression compared to Ta tumours ($P < 0.01$). There was no significant difference in

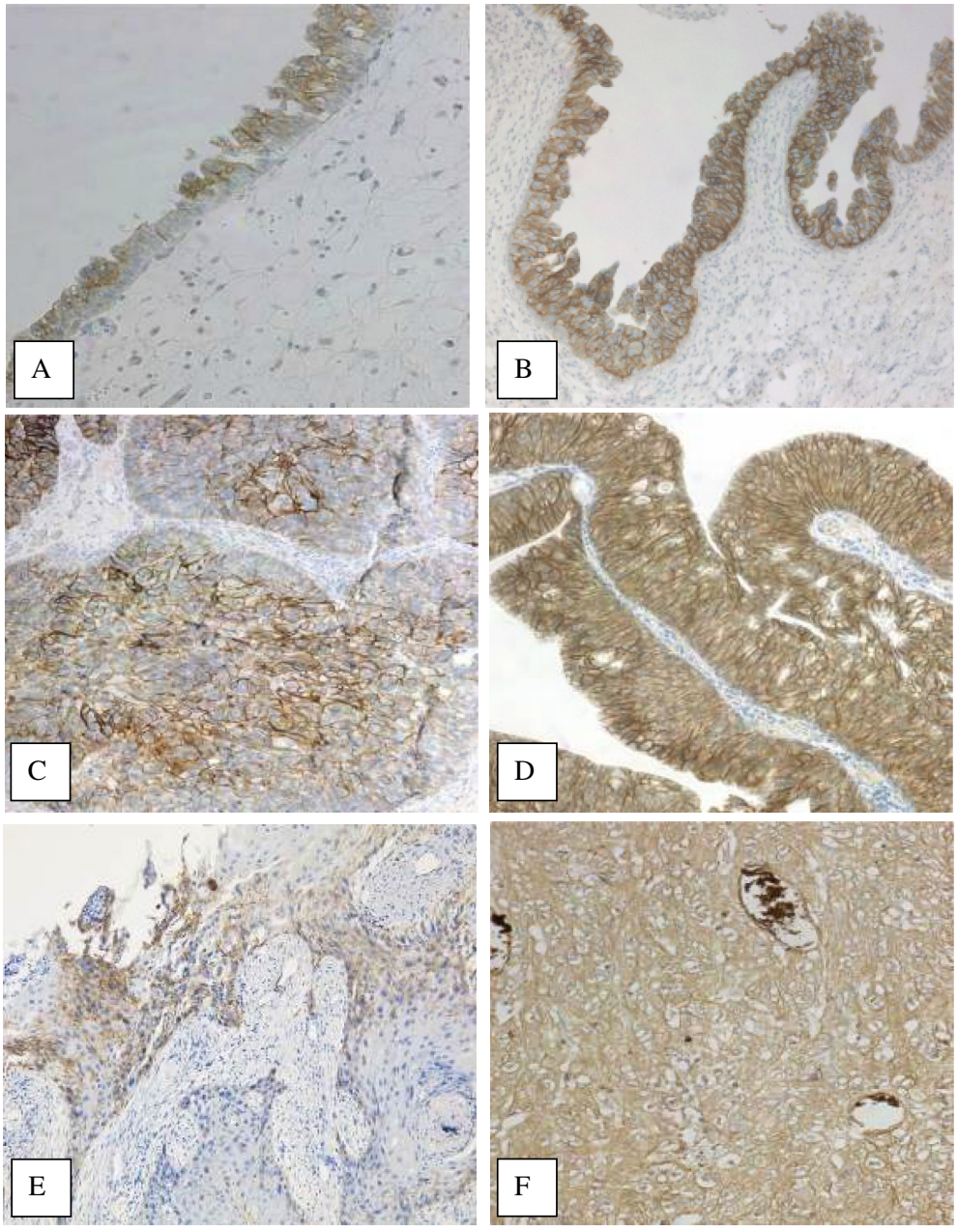


**Figure 1** IHC staining of the study group tissues. (A) A control showing negative HER2 protein, scoring 1+ in the urothelial lining. (B) A case of polypod cystitis showing negative HER2 protein immunoexpression, score 2+, IHC, Her2, DAB, ×200. (C) A case of poorly differentiated urothelial TCC, showing equivocal HER2 protein immunoexpression in >30% of tumour cells; IHC, Her2, DAB, ×400. (D) A case of papillary urothelial carcinoma showing strong HER2 protein immunoexpression, score 3+ in >70% of cells; IHC, Her2, DAB, ×200. (E) A case of moderately differentiated schistosomal-associated SCC, strongly positive for HER2 protein, score 3+; IHC, Her2, DAB, ×400. (F) A case of moderately differentiated SCC, grade II, showing negative HER2 protein immunoexpression, score 1+, IHC, Her2, DAB, ×200.

HER2 immunoexpression in different stages of SCC (Table 1).

High-grade TCC had statistically significantly greater positive HER2 immunoexpression than low-grade tumours ($P < 0.01$). There was no significant difference in HER2 expression between low-grade and high-grade malignancy in SCC.

*HER2/neu gene expression using FISH*

All control and cystitis cases with or without schistosomal infestation were negative for HER2 expression. Both TCC and SCC had a statistically significantly greater HER2 expression than control and cystitis cases (both $P < 0.01$).

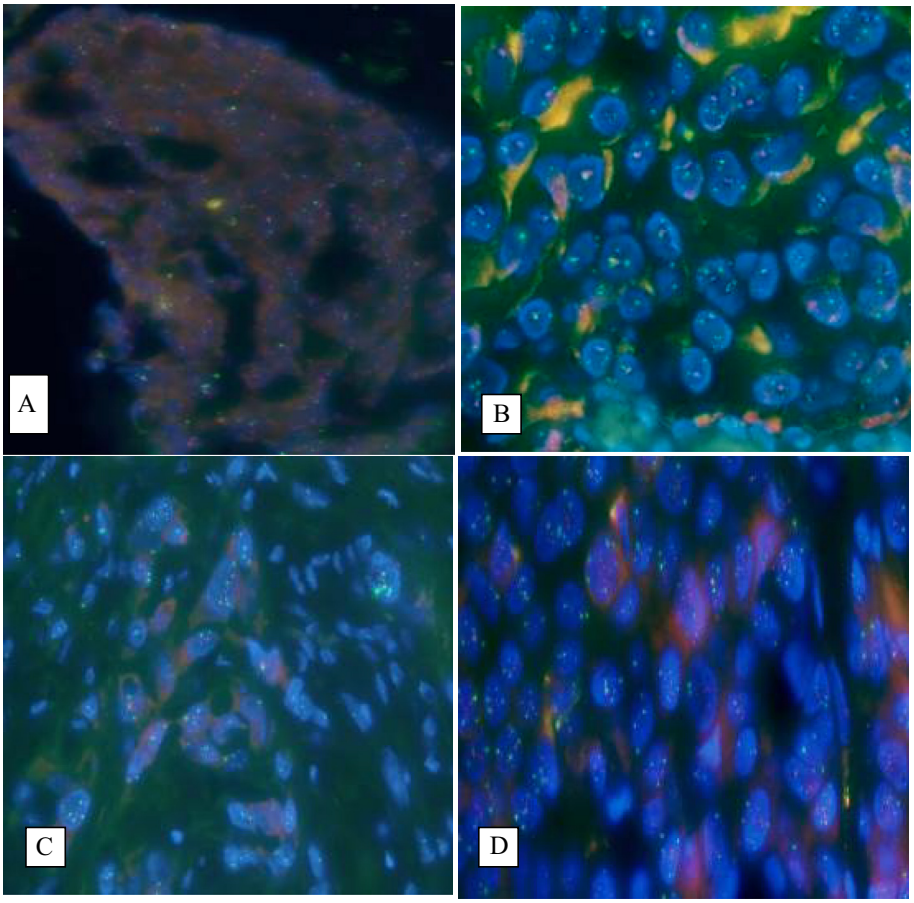


**Figure 2** HER2 amplification by FISH. (A) Papillary urothelial TCC, negative for HER2 gene amplification. (B) Urothelial TCC, positive for HER2 gene amplification, showing red clusters. (C) Bilharzial SCC, negative for HER2 gene amplification. (D) SCC, positive for HER2 gene amplification, showing >6 red signals per cell. All ×60.

HER2 expression was positive in 33% of TCC (Fig. 2A and B), compared with only 27% positive by IHC staining ($P < 0.05$), and was similar for SCC (Fig. 2, C and D), with HER2 expression of 37% by IHC staining but 53% by FISH ($P < 0.01$).

There was positive HER2 expression detected by FISH in 1/13, 3/7 and 7/13 Ta, T1 and T2–3 TCC tumours, respectively. All low-grade TCC were negative for HER2 expression using FISH and IHC staining, but there was statistically significantly greater HER2 expression detected using FISH in high-grade TCC.

## Discussion

In 2010, bladder cancer was responsible for about 170,000 deaths world-wide [18]. In the Egyptian population, bladder cancer accounts for 29.8% of all malignant diseases and it is the most common cancer among males, with schistosomal infestation previously being the most important predisposing factor, although currently the incidence of bilharzial-associated bladder cancer is declining [3].

The behaviour of cancer is now considered to be a genetically controlled process affecting cell physiology and its interaction with the host organism. It is now possible to evaluate these genetic abnormalities, study oncogenes and tumour-suppresser genes, and changes in cellular molecules, using several diagnostic and prognostic markers.

HER2/neu (c-erb-B2) is an oncogene present on chromosome 17q21, and is a transmembrane protein characterised by an extracellular domain that interacts with various growth factors. It has been shown to be expressed in several malignancies, including lung, stomach and breast adenocarcinoma [8–11]. The incidence of overexpression of HER2/neu in bladder cancer is one of the highest among all human malignancies, at 9–34% of cancers tested [18].

The success of trastuzumab therapy in patients with breast carcinoma has stimulated interest in exploring its antitumour activity in patients with bladder cancer, although there is controversy about the value of HER2 expression in these patients [11,12]. Early studies showed increased HER2 expression and with both higher tumour

stage and grade. Others suggested that the overexpression of HER2 was an independent variable in determining patient survival [19]. HER2 overexpression was evaluated using IHC, FISH and blood analysis. Trastuzumab therapy in patients with breast cancer requires candidates to show either 3+ overexpression by IHC or 2+ overexpression by IHC with positive FISH results [20].

Schistosomal infestation is considered to be one of the predisposing factors for the development of urinary bladder cancer. Bladder cancers diagnosed in areas of endemic schistosomiasis are usually SCCs, but it appears that the histopathology of bladder cancer in these areas is changing. In a recent study of schistosomiasis-associated bladder cancer conducted in Egypt, the proportion of TCC increased over time [21].

In the present study HER2 was not identified in benign and inflammatory urothelium by both IHC staining and FISH. Schistosomal infestation seemed not to affect the gene expression. HER2 expression by IHC staining was positive in 30% of all malignant cases, 27% of TCCs, and 37% of SCCs, as was found by Abd-El-moneim et al. [22].

Positive HER2 expression was statistically significantly greater in high-stage/grade TCC tumours than in low-stage/grade tumours; this is in accord with the results of Hansel et al. [23], who found over-expression of HER2 protein in invasive metastatic disease.

Using FISH, 40% of all malignant cases were positive for HER2 expression, as were 33% of TCCs and 52% of SCCs. Our results consolidate those of Fleischmann et al. [24], who concluded that for better patient selection, the FISH technique is more accurate than IHC staining.

The present study has several limitations; it included only a few controls and patients with cystitis, the metastatic status of the patients was not recorded, and the effect of the previous use of intravesical installation (e.g., BCG) in patients with NMI TCC on HER2 expression was not considered.

In conclusion, HER2 protein is expressed in a subset of patients with bladder cancer who appeared to have a more aggressive disease than those having HER2-negative tumours, and who might be candidates for anti-HER2-targeted therapy as is already used in other malignancies. FISH has a higher detection rate for HER2-positive tumour than does IHC staining.

## Conflict of interest

None.

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None.

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