Her 2/neu Gene and VEGF in Bladder Cancer in Egypt: Relationship to Schistosomiasis

Olfat A. Hammam [1], Iman Abdel Aziz [2],Ola Mahmoud [2], Manal Zahran [2], Amr Alkholy [3], Ahmed Abdel Hadi[1], Maha Akl [1], Mohamed Wishahi [3]. Bruno Voss[4] and Sabine Boehm[4]

Departments of Patholog [1], Hematology [2], Urolog[3], Theodor Bilharz Research Institute, El-Nil Street, Giza, Egypt
Research Institute for Occupational Diseases[4], Ruhr University Bochum, Germany
*totoali1@hotmail.com

Abstract: The aim of the current study was to assess Her2/neu protein on paraffin tissue sections and serum VEGF in carcinoma of the urinary bladder in a cohort of Egyptian patients. Furthermore, they were correlated to the schistosomal-associated and non-schistosomal associated bladder cancer as well as tumor types and disease stages. Immunohistochemical (IHC) procedure for Her 2/neu, FISH for detection of Her2/neu gene and serum level of VEGF by EIISA were performed in 35 patients with chronic cystitis (10 patients with nonschistosomal chronic cystitis and 25 patients with chronic schistosomal cystitis), 135 were schistosomal-associated malignant patients (75 cases of squamous cell carcinoma and 60 cases of urothelial carcinoma) and 50 cases of non schistosomal-associated urothelial carcinoma. In addition to 20 normal blood donor volunteers act as control. IHC results for Her 2/neu was overexpressed in malignant group compared to control and chronic schistosomal cystitis groups ($p<0.01$). In malignant group it was 1^+ in 33 (30%), 2^+ in 45 (40.9%) and 3^+ in 32 (29.09%). Her 2/neu incidence was significantly higher in urothelial carcinoma group 80/110 (72.2%) compared to SCC 30/75 (40%) with ($p<0.01$) and in high grade tumors than low grade tumors with ($p< 0.01$). FISH results in SCC showed that the signal ratio were 0-1.0 in 2 (6.6%), 1.1-2.0 in 18 (60%), and ≥2.0 in 10 (33.35%), which were considered positive for Her 2/neu gene amplification. In urothelial carcinoma the signal ratio was 0-1.0 in 10 patients (12.5%), 1.1-2.0 in 25 (22.3%), and ≥2.0 in 45 (58.2%). Overexpression of Her2/neu gene was significantly higher in high grades; 31 (63.6%) than in low grades; 14(56%) tumor with ($p< 0.01$), also Her2 /neu gene was significantly ($p< 0.01$) higher in invasive tumors; 45 cases (78.9%) than in non invasive tumors 10 (43.3%) with high significance ($p< 0.01$). The serum VEGF levels showed higher levels for SCC, urothelial carcinoma patients, chronic cystitis patients compared to normal controls, they were 94.7% (71/75), 89% (98/110), and 22.9% (8/35), 5% (1/20) respectively. Our results suggest that Her 2/neu overexpression might provide additional prognostic information in patients with bladder carcinomas. Because 50% of our patients harbor Her 2/neu overexpressing those patients may potentially benefit from molecular targeted therapy targeting Her 2/neu for bladder carcinoma and they should be identified by gene amplification analysis using FISH in IHC 2+ patients. In addition association between increased serum VEGF levels with high grades and invasive bladder cancer patients indicates that serum VEGF may play a role in the invasion and metastasis of cancer and may serve as an indicator of tumor progression and future recurrence and may be a candidate as a new noninvasive diagnostic tool.

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1. Introduction:

In Egypt, bladder cancer accounts for about 30% of all cancers, where it is the most common malignancy in men and the second most common malignancy in women after breast cancer (El-Mawla *et al.* 2001, Jemel *et al.* 2005). It has been associated with many pathogenetic factors most commonly bilharzial infestation, which is an endemic infection in the Nile River Valley (El Sebaie *et al.* 2005). In countries with a long history of schistosomiasis, research studies have identified the following risk factors for infection with S haematobium: male gender, an age < 20 years, living in smaller rural communities, exposure to canal waters, reagent strip detected hematuria and proteinuria, and a history of burning micturation (El-Harvey *et al.* 2000, Gabar *et al.* 2000). Most investigators have accepted the association between schistosomiasis and bladder cancer since the work of Ferguson 1911. The development of bladder cancer in a younger age group affects males more and is usually associated with schistosomal infection. Also it is accompanied with a high mortality rate and clinicopathologic features of schistosomal-associated bladder cancer (SABC) (Mostafa *et al.* 1999). The high frequency of squamous cell carcinoma (SCC) is due to schistosomiasis-infested bladders that frequently show squamous metaplasia and dysplasia of the

transitional epithelium (El Bolkainy *et al.* 1981). Recently, however, a relative increase in the frequency of transitional cell type in schistosomiasis-associated bladder cancer has been noted (Gouda *et al.* 2007, Felix *et al.* 2008, Heyns and van der Merwe 2008). The neoplastic changes in the urothelium of bladder are a multistep phenomenon (Carroll 1995). The exact genetic events leading to urothelial transformation involve the activation of oncogenes, inactivation or loss of tumor suppressor genes and alterations in the apoptotic gene products (Sandberg and Berger 1994).

The Human epidermal growth factor receptor 2 (Her 2/neu) oncoprotein, also known as (NEU, EGFR2, or ErbB2) 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), is known to contribute to physiological mechanisms of cell proliferation by intrinsic tyrosine-kinase-activity (Wülfing *et al.* 2005). Binding of ligands, such as epidermal growth factor and transforming growth factor alpha (TGFα), to their extracellular ligand-binding domain initiates intracellular signaling cascades, leading to progression, proliferation, migration and survival of cancer cells (Yarden and Sliwkowski 2001).

EGFR and Her 2/neu are dysregulated in many human cancers (Slamon *et al.* 1978). Recent studies indicate the role of Her2/ neu in the development of numerous types of human cancer. Her 2/neu overexpression and/or amplification have been detected in a variety of cancer types, including non–small cell lung cancer, pancreatic carcinoma and gastric carcinoma (Gravalos and Jimeno 2008), colonic carcinoma (Schuell *et al.* 2006) ovarian carcinoma (Des Guetz *et al.* 2006, Tabernero 2007), bladder cancer (Eltze *et al.* 2005) and 10%-34% of invasive breast cancers and correlates with clinical outcome, poor prognosis, and constitute a predictor factor of poor response to chemotherapy and endocrine therapy. Her 2/neu overexpression is associated with shortened disease-free and overall survival compared with patients who have Her 2/neu-negative tumors (Kaptain *et al.* 2001).

Superficial bladder cancer (SBC) is a precursor of muscle-invasive, potentially life-threatening bladder cancer. Given the field cancerization effect and the risk of recurrence and progression, SBC appears the most suitable target for bladder cancer systemic chemoprevention. However, new risk biomarkers are demanded to select at-risk subjects and to conduct efficient clinical chemoprevention trials. Angiogenesis represents a key step for tumor progression and metastatic spread in solid tumors. Vascular Endothelial Growth Factor (VEGF) is the most known angiogenic factor and is involved in early stages of bladder carcinogenesis (Nicholson *et al.* 2001). Also expression of VEGF either as tissue or soluble form, has been reported to have prognostic significance in several cancers (Mohammed *et al.* 2007). Overexpression of Tyrosine–kinase receptors, including VEGF, and Her 2/neu, has been associated with the progression of cancer and poor prognosis in urothelial tumors (Bolenz and Lotan 2008). It is worth mentioning that all of these studies were limited to transitional cell carcinoma which was not schistosomiasis-associated. The aim of the current study was to assess Her 2/neu protein and gene and soluble VEGF in carcinoma of the urinary bladder in a cohort of Egyptian patients, and both markers were correlated to the schistosomal status as well as tumor types and disease stages.

2. Materials and methods

Materials:

This study was conducted on 220 patients (156 males and 64 females having mean age of (65.5± 11.2) patients admitted to the Urology Department at Theodor Bilharz Research Institute (TBRI) Hospital. In addition, 20 age and sex matched healthy subjects as a blood donor control group and 5 cases of cystoscopic biopsies during prostatectomies as control group for IHC & FISH; upon patient's consent were included in this investigation. Tumor specimens were taken by cystoscopy (Transurethral resection (TUR) biopsies) and cystectomies. Only cystoscopic biopsies containing muscle tissue were included, so that muscle invasion by the tumor could be assessed. The study protocol was approved by the ethical committee of TBRI according to the institutional committee for the protection of human subjects and adopted by the 18th world medical assembly, Helsinki, Finland and informed consent from all patients underwent cystoscopy and biopsy from apparent growth and lesions was taken. Patients were subjected to full clinical examination, routine laboratory investigations, complete urine analysis, abdominal and pelvic ultrasonography, general and abdominal examination, digital rectal examination (DRE), bimanual examination under anesthesia, plain x-ray of the urinary tract, intravenous urography (IVU), cystoscopy and TUR biopsies were taken from apparent growth. Accordingly they were grouped into:

- Group I: Five male patients (age range 25-50), with normal bladder urothelium, subjected to prostatectomy served as normal controls after taking their consent.
- Group II: Thirty five patients with chronic cystitis classified as:

- 10 patients with nonschistosomal chronic cystitis.
- 25 patients with chronic schistosomal cystitis, which include:

12 patients with dysplastic changes, 4 patients with squamous metaplasia and 9 patients with simple chronic schistosomal cystitis.

- Group III: One hundred and eighty five malignant patients:

- 135 were schistosomal-associated include 75 cases of SCC and 60 cases of schistosomal-associated urothelial carcinoma.
- 50 cases of non schistosomal-associated urothelial carcinoma.

Urothelail carcinoma (110 cases): Are categorized according to pathological stages into: 40 Non invasive tumors (pTa+pT1) and 70 invasive tumors (pT2+pT3) and according to pathological grades into: 46 low grade urothelial carcinoma and 64 high grade urothelial carcinoma.

Methods:

Histopathological Study

Tissues were fixed in 10% buffered formalin, paraffin embedded and processed routinely. Hematoxylin and Eosin stained slides were used to evaluate the pathological diagnosis of all bladder lesions and to assess urothelial carcinoma [transitional cell carcinoma (TCC)] cases for pathological grades according to World Health Organization (WHO) (Mostofi *et al.* 1988) and pathological tumor stage, in accordance with WHO Classification of tumors (Eble *et al.* 2004). Diagnosis of schistosomal infestation was based on detection of Schistosoma eggs in tissues and/or detection of circulating Schistosoma antibodies in sera of patients by enzyme linked immunosorbent assay (ELISA).

Immunohistochemical (IHC) procedure for Her 2/neu

For IHC, a standard 3-layer protocol was used, as previously described by

Hsu and Raine (1981). Unstained sections were processed for immunostaining with Her 2 monoclonal antibody, Endogenous peroxidase was blocked by 0.3% hydrogen peroxide for 30 minutes. The antibody-binding epitope of the antigen was retrieved by microwave treatment for 15 minutes in citrate buffer at 700°C. We used Her 2 primary antibody at a dilution of 1:50, and incubated for 24 hours in a humid chamber (Dako, Copenhagen, Denmark). The sections were then incubated with the secondary biotinylated antibody followed by avidin peroxidase complex according to the manufacturer's instructions (Universal Detection Kit, Dako, Denmark). A brown color was developed with 3,3 diaminobenzidine for 5 minutes, and counterstained with Mayer's hematoxylin. Negative controls in which the primary antibody was omitted and replaced by phosphate buffered saline were also used. Breast cancer known to express Her 2 /neu was used as a positive control.

Urothelial cells in entire sections were examined in ten consecutive fields under light microscopy at magnifications x 400 with the highest expression and the percentage was calculated from their mean. A negative staining was defined as the absence of cells expressing the marker (zero %). The intensity of reactivity was scored according to Ooi *et al.* (2004).

Fluorescence in situ hybridization (FISH) to detect Her 2/neu gene

The technique of FISH to detect Her 2/neu gene was performed according to Matsubara *et al.* (2008), after usual processing for remove of wax and hydration of the slides, we use enzymic digestion by 5 µg/ml proteinase K (Boehringer Mannheim; Mannheim, Germany) in 0.1 M Tris-HCI (pH 8.0) containing 50 mM EDTA. Disodium salt for 45 minutes at 45°C before incubation with the slides. Then incubate slides with DIG – labelled DNA probe (Zymed Lab-Sa system, USA) for 10 minutes at 95°C. Then incubate slides in humid chamber at 37°C for 45 minutes with mouse anti DIG, then add on slides goat anti mouse Cy3 at 3°C for 45 minutes. Put DAPI at 37°C for 60 seconds at 37°C for nuclear stain (blue nucleus). To preserve fluorescent labelling, sections were mounted using DABCO antifade (Sigma, Missouri, USA). Each section was mounted in approximately 5 µl of mounting medium and the slide cover is sealed with entallin. In each preparative run, positive controls (Probe Check control slides supplied by the manufacturer) were included.

An Olympus BX 61 microscope equipped with appropriate filters for DAPI, Spectrum Green and Spectrum Orange was used to score the number of signals per nucleus under magnification power x600. Images were captured using a CCD digital camera and Quips FISH imaging software (Meta Cyte scanning image cytometer, Meta Systems, Altlussheim, Germany). The number of fluorescent signals was counted in 60 urothelial cell in each case. The mean number of signals per nucleus was determined.

Serum VEGF was done using EIA technique:

From the studied subjects five milliliter of venous blood samples were collected under complete aseptic conditions, delivered into a clean tube and left to clot. Serum was separated by centrifugation at 3000 rpm for 15 min. , sera were separated and divided into small aliquots for assay of liver function tests {serum bilirubin, alanine

aminotransferase (ALT), aspartate aminotransferase (AST) and serum albumin}, kidney function tests (serum creatinin and blood urea) using autoanalyzer (Hitachi 736, Hitachi, Japan) and Determination of serum level of VEGF using quantitative sandwich enzyme immunoassay technique (ELISA). (using R&D system, USA).

Statistical Analysis

For statistical analysis, Statistical Package for the Social Sciences (SPSS, Chicago, IL, USA) version 10.0 for Windows software was used. The chi-square test was used to detect statistically significant differences between the groups, with a significance level of ($p<0.05$). Comparison between means of different groups was done using one way ANOVA. Comparison between percent of positive cases was calculated by Chi-square test.

3. Results

Clinico-pathological data and immunostaining results for the cases exhibiting overexpression of Her 2/neu are illustrated in (Table 1).

Assessement of Her 2/neu protein using IHC technique

In control cases no Her 2/neu expression was detected. Her 2/neu- positive immunostaining was limited to the cell membrane. It was overexpressed ($p<0.01$) in malignant group compared to control and chronic schistosomal cystitis groups. In malignant group it was 1^+ in 33 (30%), 2^+ in 45 (40.9%) and 3^+ in 32 (29.09%). Her 2/neu incidence was significantly ($p<0.01$) higher in urothelial carcinoma group 80/110 (72.2%) compared to SCC 30/75 (40%).

On classifying urothelial carcinoma cases on the basis of their histopathological grades into low and high grades; the numbers of cases showed overexpression of Her 2/neu were found to be significantly ($p< 0.01$) higher in high than low grade tumor.

On classifying urothelial carcinoma cases on the basis of their histopathological stages into non muscle invasive and muscle invasive urothelial carcinoma; we found Her 2/neu overexpressed in ($p< 0.01$) 23/40 (55.5%) in non muscle invasive urothelial carcinoma compared to 57/70 (81.4%) (Table 1, Figs 1A &B & C).

Assessment of Her 2/neu gene using FISH technique

In primary tumors, in SCC the signal ratio was 0-1.0 in 2 patients (6.6%), 1.1-2.0 in 18 (60%), and ≥2.0 in 10 (33.35%)., which was considered positive for Her 2/neu gene amplification. In Urothelial carcinoma the signal ratio was 0-1.0 in 10 patients (12.5%), 1.1-2.0 in 25 (22.3%), and ≥2.0 in 45 (58.2%).

According to the classification of grade, the number of cases showed over- expression of Her 2/neu gene were found to be significantly ($p< 0.01$) higher in high 31 (63.6%) than low grade 14(56%) tumor.

According to the classification of stage, we found Her 2/neu overexpressed ($p< 0.01$) in 10(43.3%) non muscle invasive urothelial carcinoma compared to those with muscle invasive 45(78.9%) (Table 1, Figs 2A&B&C).

Determination of positive VEGF rates

According to the mean level of VEGF in normal human serum (123.53 pg/ml),the normal level of VEGF was calculated as 227.20 pg/ml. Taking this as the standard, the VEGF expression positive rates for SCC, urothelial carcinoma patients, chronic cystitis patients and normal controls were 94.7% (71/75), 89 % (98/110), and 22.9% (8/35), 5% (1/20) respectively, (Table 2).

Relationship between VEGF and Schistosomiasis:

Of the 35 chronic cystitis patients, 10 were found without schistosomal infestation and 25 were chronic cystitis with schistosomiasis. There was a significant ($P< 0.01$) difference in serum VEGF level between the two groups. Of the 60 patients with schistosomal associated urothelial carcinoma, the positive rate of VEGF was 91.7% (55/60), whose VEGF level (584.77± 443.87 pg/ml), which was significantly ($P< 0.01$) higher than that of non schistosomal associated urothelial carcinoma where the positive rate of VEGF was 84% (42/50) and VEGF level was (429.41± 289.83 pg/ml) (Table 3).

Relationship between VEGF and histopathological grades

According to histopathological grades, 46 patients were classified as having low grade tumor with a VEGF level of 329.68 ± 228.45pg/ml. Sixty-four patients were classified as having high grade tumor with a VEGF level of 558.42± 370.10 pg/ml. In the two groups, the serum level of VEGF expression was significantly ($P< 0.001$) higher in histopathologically higher grade tumors, compared to lower grades, and there was a positive correlation between the serum level of VEGF and tumor grades (Table 3).

Relationship between VEGF and Histopathological stages

Superficial bladder cancer (SBC) is a precursor of muscle-invasion, potentially life-threatening bladder cancer. In this study, the levels of VEGF in the patients with non muscle invasive-

urothelial carcinoma were significantly (P< 0.001) lower than those with muscle invasive-urothelial carcinoma (418.21 ± 243. 25 pg/ml and 550.10 ± 436.60 pg/ml respectively). This finding suggested that high-level expression of VEGF predicts a higher level of muscle invasiveness and higher metastastatic tendency of the tumor (Table 3).

Table 1: Expression of Her2/neu protein using IHC and Her2/neu gene using FISH in the bladder tissue in the studied cases

Parameters	No of positive cases with Her2/neu in bladder tissue by IHC No (%)	No cases with normal expression of Her2/neu gene by FISH	No cases with over-expression of Her2/neu gene by FISH No (%)
Control (n=5)	0 (0%)	5 (100%)	--
Chr. cyst (n=35)	4 (8%)	13(86.6%)	2 (13.4%)
Ch non Schist. Cyst (n-10)	0 (0%)	5 (100%)	---
Ch Schist. Cyst (n=25)	4 (8%)	8 (80%)	2 (20%)
Malignant lesions (n=185)	**110 (59.4%)** $^{*,\wedge}$		55 (50%)$^{\wedge}$
SCC (n=75)	**30 (40%)** $^{*,\wedge}$		10(33.3%)$^{\wedge}$
Urothelial carcinoma (n=110)	**80 (72.2%)** $^{*,\wedge,\#}$		45(58.2%)$^{\wedge,\#}$
* Non Sch.Ass. urothelial carcinoma (n=50)	30 (60%)$^{*,\wedge,\$}$		13(43.3%)$^{\wedge}$
* Sch Ass.urothelial carcinoma (n=60)	50 (83.3 %)$^{*,\wedge}$		32(68%)$^{\wedge,\$}$
Histopathological grades:			
Low grade (n=46)	25 (54.3%)		14(56%)
High grade (n=64)	55 (85.9)$^{¥}$		31 (63.6%)$^{¥}$
Histopathological stages:			
Non muscle invasive urothelial carcinoma (n= 40)	23 (55.5%)		10(43.3%)
Muscle invasive urothelial carcinoma (n=70)	57 (81.4 %)$^{€}$		45(78.9%)$^{€}$

* P < 0.01 compared to control group.
$^{\wedge}$P < 0.01 compared to chronic schist. cystitis group.
$^{\#}$ P < 0.01, compared to SCC.
$^{\$}$ P < 0.05 compared to Sch TCC respectively.
$^{¥}$ P < 0.01 compared to low grade tumor .
$^{€}$ P < 0.01 compared muscle invasive urothelial carcinoma .
Chr. cyst = chronic cystitis
Ch Schist.Cyst = chronic schistosomal cystitis
Ch non Schist.Cyst = chronic non schistosomal cystitis SCC= squamous cell carcinoma
Schistosomal associated urothelial carcinoma =Sch ass. urothelial carcinoma

Table 2: Serum positive expression of VEGF in different groups

Group	n	VEGF expression	
		Negative (%)	Positive (%)
Normal Control	20	19 (95)	1(5)
Chronic Cystitis	35	27 (77.1)	8 (22.9)
SCC	75	4 (5.3)	71(94.7)
Urothelial Carcinoma	110	12 (11)	98(89.0)

Table 3: Relationship between serum expression of VEGF (pg/ml) and pathological characteristics of Urothelial Carcinoma

Pathological characteristics	N	VEGF expression	
		mean± SD	*p*
Sch.Ass. urothelial carcinoma	60	584.77± 443.87	
Non Sch.Ass. urothelial carcinoma	50	429.41± 289.83	0.016
Low grade	46	329.68 ± 228.45	
High grade	64	558.42± 370.10	0.001
Muscle invasive urothelial carcinoma	70	550.10 ± 436.60	
Non muscle invasive urothelial carcinoma	40	418.21 ± 243. 25	0.001

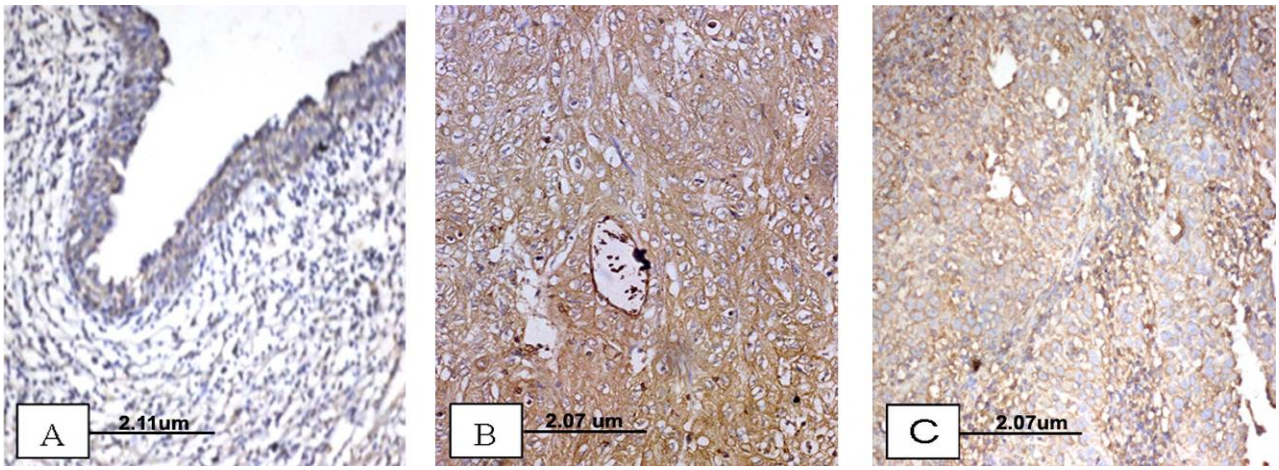

Figure 1: A) A case of chronic schistosomal cystitis, showing mild memranous expression of Her 2, involving the whole layers of the urothelium (IHC, Her2, DAB, x20). B) A case of schistosomal-associated moderately differentiated squamous cell carcinoma showing moderate membranous expression of Her 2 in about 70% of the malignant squamous cells (IHC, for Her 2 DAB, x40). C) A case of invasive urothelial cell carcinoma, high grade, showing marked membranous expression of Her2 in the majority of malignant urothelial cells (IHC for Her-2, DAB, x40).

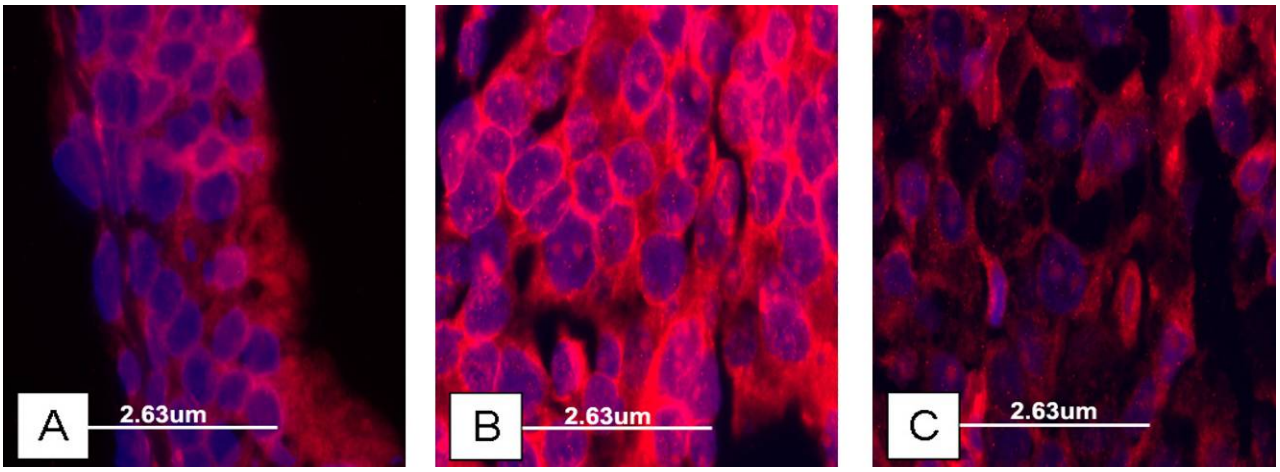

Figure 2: A) A case of chronic schistosomal cystitis, showing mild number of red signals in the nuclei of urothelial cells for Her 2 gene in the urothelium (FISH, Her2 gene, x60). B) A case of schistosomal-associated moderately differentiated squamous cell carcinoma showing moderate number of red signals in the nuclei of urothelial cells for Her 2 gene in about 50% of the malignant squamous cells (FISH, Her 2 gene x60). C) A case of invasive urothelial cell carcinoma, high grade, showing marked red signals in the nuclei of urothelial cells for Her 2 gene the majority of malignant urothelial cells (FISH, Her-2 gene, x60).

4. Discussion:

Bladder cancer is a major health problem in Egypt. The two major types of bladder cancer in Egypt are bilharzial and non-bilharzial bladder cancer. The present study included 135 bladder cancer patients infected with bilharziasis and revealed high VEGF levels compared to non-bilharzial cases. Recently, bilharzial bladder cancer was found to be positively correlated with over- expression of Bcl-2 (apoptotic marker) (Swellam *et al.* 2004a) that enhance the angiogenesis process and induced increase of VEGF protein secretion as reported by Biroccio *et al* (2000). These findings may hypothesized that molecular changes occurring in bladder cancer patients infected with bilharziasis can undergo the phenotypic (angiogenic) switch therefore able to induce phenotypic changes in endothelial cells, leading to angiogenesis (Swellam *et al.* 2004a). Our study reported significantly higher VEGF levels in SCC compared to TCC types. Previously, it was reported that SCC components are more genetically unstable and had alterations not present in TCC cases (Swellam *et al.* 2004b). Accordingly, it is possible to postulate that SCC of the bladder stimulates angiogenesis by directly secreting angiogenic substances or by activating and releasing angiogenic compounds stored within the extracellular matrix (Campbell 1997). Moreover, significantly high levels of VEGF were observed in high grade compared to low grade bladder tumors, suggesting that VEGF production increases as tumors become more anaplastic. Shinoda *et al.* (1999) and Tuttle *et al.* (2002) observed similar findings in other tumors. Our result also revealed significant association between increased VEGF levels and tumor invasion. Association between increased VEGF levels with high grade and tumor invasion in bladder cancer patients indicates that VEGF may play a role in the invasion and metastasis of cancer and may serve as an indicator of tumor progression and future recurrence.

Oncogene amplification is a common mechanism of disease progression in many solid tumors and it may be used as a prognostic marker for aggressiveness of growth and behavior in some of these malignancies (Menard *et al.* 2001).

Studies have shown that Her 2/neu overexpression induces cell transformation and that Her2/neu positive tumors are more aggressive (Eltze *et al.* 2005). With regard to the distribution of Her 2/neu in normal tissues, Her 2/neu is slightly expressed only in the liver, bile duct, gastrointestinal tract, skin, genital organs and urinary tract, with limited expression in most normal tissues (Natali *et al.* 1990, Matsubara *et al.* 2008). The methods to analyze Her 2/neu in tissues include analysis of gene amplification, mRNA overexpression, and protein overexpression; however, possible methods for use on formalin-fixed paraffin sections are IHC and FISH. It has been indicated that examination of gene amplification rather than antigen expression is a more reliable method to identify patients with Her 2/neu positive breast cancer (Mass *et al.* 2005, Matsubara *et al.* 2008).

In the present study, IHC results for Her 2/neu was overexpressed ($p<0.01$) in malignant group compared to control and chronic schistosomal cystitis groups. In malignant group it was 1+ in 33 (30%), 2+ in 45 (40.9%) and 3+ in 32 (29.09%). Her 2/neu incidence was significantly ($p<0.01$) higher in urothelial carcinoma group 80/110 (72.2%) compared to SCC 30/75 (40%). While Her 2/neu overexpression was observed in 12% (Lipponen *et al.* 1991) to 71% of urothelial cancers (Gandour-Edwards *et al.* 2002) it correlated with grade and stage in some studies (Miyamoto *et al.* 2000) but not in others (Ioachim *et al.* 2000). Several hypotheses could explain these wide variations, as well as the relatively low rate of Her 2 overexpression reported here. One of the major issues is the variability in IHC assays, related to the heterogeneity between kits, antibodies, protocols, interpretations or cut-off values, in their study performing this analysis using both IHC staining and FISH, Her 2/neu overexpression was observed in 33.3% (10), 58.2% (45) of SCC and urothelial carcinoma respectively (Lae *et al.* 2010) while Aly and Khaled, (2004) found that 9/21 (43%), and 3/16 (19%) of cases with squamous and transitional cell carcinoma had C-erb-B2 gene amplification, respectively.

In the present study, overexpression of Her2/neu gene was significantly ($p< 0.01$) higher in high 31 (63.6%) than low grade 14(56%) tumor and in high than low grade tumor with ($p< 0.01$, we found that overexpression correlate with grade, and stage, while Aly and Khaled, (2004), found that no significant association was observed between C-erb-B2 amplification and tumor grade. They suggest that relative C-erb-B2 gene amplification is associated with aggressive bladder cancer and may play an important role in tumor progression.

On comparison between IHC and FISH, Sauter *et al.* (1993) reported that Her 2/neu gene amplification was detected in only 7% (10/141) of patients with urothelial cancer (36 pTa, 42 pT1, 67 pT2-T3/20 G1, 39 G2, 46 G3 and 6 with grade and stage unknown), whereas 43% (61/141) of tumors were Her 2/neu positive by IHC. In addition, Kruger *et al.* (2002) studied 203 patients with urothelial cancer and reported that 37% (76/203) of patients were Her 2/neu positive by IHC, whereas Her 2/neu gene amplification was detected in only 5% (2/42) of

patients. Moreover, de Pinieux *et al.* (2004) reported that 23% (15/64) of patients with invasive urothelial cancer were Her 2/neu positive, while Her 2/neu gene amplification was detected in 28% (6/21) of patients.

In the current study, FISH results in SCC Her 2/neu gene amplification, was 10 (33.35%). In Urothelial carcinoma Her 2/neu gene was overexpressed in 45 (58.2%). Lae *et al.* (2010), found Her 2/neu overexpression in 9.2% of tumor samples. In comparison of Her 2/neu expression between IHC and FISH, it was suggested that the dissociation between gene amplification and overexpression could be related to a point mutation that leads to protein overexpression, translocation or transcriptional up regulation (Sauter *et al.* 1993)

Evidence from breast cancer indicates that only tumors with Her 2/neu gene amplification respond to an anti-Her 2/neu targeted therapy, such as trastuzumab. Using the same principle, 78% of muscle-invasive urothelial bladder carcinomas should be suitable for such treatment. The potential involvement of Her /neu in the proliferation of urothelial carcinoma led to the initiation of anti-Her 2/neu targeted therapy protocols in advanced disease. Single-agent data with trastuzumab in urothelial cancer are not available or limited to case reports (Peyromaure *et al.* 2005). The efficacy of molecular targeting therapy for various molecules including EGFR/VEGF/Her 2/neu has been proved clinically in a wide range of cancers (Yoon *et al.* 2004).

In the running study we found that Her 2/neu protein was expressed in 68% of cases of schistosomia associated urothelial carcinoma compared to 43.3% in nonschistosoma associated urothelial carcinoma, also in SCC in 33.3% of the cases. The study examined the prognostic value of C-erb-B2, among other markers, in bilharzial related bladder cancer (Haitel *et al.* 2001), has also suggested that C-erb-B2 expression was associated with poor prognosis.

5. Conclusion:

Her 2/neu overexpression might provide additional prognostic information in patients with muscle-invasive bladder carcinomas; Because 78.9% of our patients harbor Her 2/neu overexpressing those patients may potentially benefit from molecular targeted therapy targeting Her 2/neu for invasive bladder carcinoma and they should be identified by gene amplification analysis using FISH in IHC 3+ patients. In addition, serum VEGF levels are increased in patients with bladder carcinoma and may be a candidate as a new noninvasive diagnostic tool.

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Corresponding author

Olfat A. Hammam

Departments of Patholog , Theodor Bilharz Research Institute, El-Nil Street, Giza, Egypt

*totoali1@hotmail.com

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