

# **Expression of Epidermal Growth Factor Receptor and Transforming Growth Factor Alpha in Chronic Bladder Lesions**

***Thesis***

Submitted For Partial Fulfillment of Master Degree

In pathology

***By***

**Amira Kamel Abd Raboh**

*M.B., B.Ch. Cairo University*

**Supervised by**

**Prof. Dr. Ali El-Hindawi**

*Chairman of Pathology Department*

*Faculty of Medicine-Cairo University*

**Prof. Dr. Afkar Abd El-Ghany**

*Chairman of Pathology Department*

*Theodor Bilharz Research institute*

**Dr. Mostafa Khodair**

*Lecturer of Pathology*

*Faculty of Medicine-Cairo University*

Faculty of Medicine-

Cairo University

2013

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

مكتبة

## *Acknowledgement*

*First of all, I wish to express my sincere thanks to **ALLAH** for his care and generosity all through my life.*

*I am greatly honored to express my deep thanks and gratitude to **Prof. Dr. Ali El-Hidawi**, Professor of pathology, x chairman of the pathology department, and x chairman of Tumor markers unit, Faculty of medicine, Cairo University, for his continuous support and guidance, valuable suggestions, expert advice and generous help which have greatly helped me to complete this work.*

*I would like to express my deepest thanks and state great appreciation to **Prof. Dr. Afkar Abdel-Ghany**, chairman of pathology department, Theodor Bilharz Research Institute for her kind supervision, great support, encouragement and her generosity in time and effort.*

*I would like to express my sincere appreciation and deep gratitude to **Prof. Dr. Olfat Hammam** Professor of pathology Theodor Bilharz Research Institute for her continuous effort, valuable advice, great help and kind cooperation all through this work.*

*I would like to thank **Dr. Mostafa Khodair**, lecturer of pathology, faculty of medicine, Cairo University.*

*Lastly, I want to express my deep thanks to **My Parents & my husband**, without their help, kind, great support and encouragement, this work could not have been completed.*

*✍ Amira Kamel*

## ABSTRACT

### **Objectives:**

Evaluation of the expression of EGFR and TGF alpha in the urothelial cells of neoplastic and non-neoplastic urothelial lesions of the urinary bladder, and correlation with tumor grade, stage and associated bilharziasis.

### **Material and Methods:**

Fifty five different urinary bladder lesions were studied. Data concerning age, sex, tumor grade, stage, and associated bilharziasis were obtained. Each case was studied using monoclonal antibodies for EGFR and TGF alpha and examined for detection of immunostaining in urothelial cells.

### **Results:**

Bladder cancer had highest incidence of cystitis in fourth decade while of bladder cancer was in seventh decade . Tumor grade was correlated significantly with tumor stage. EGFR correlates significantly with tumor grade , stage and with bilharzial association. TGF alpha positively correlates with tumor grade , stage and bilharzial association ,but not significantly.

### **Conclusions:**

EGFR and TGF alpha overexpression in malignant cases were significantly higher than in chronic cystitis.

### **Key Words:**

EGFR , TGF alpha, urothelial bladder lesions, cystitis, carcinoma.

## *Contents*

<b>Subjects</b>	<b>Page</b>
• List of Abbreviations.....	I
• List of Tables.....	II
• List of Graphs.....	IV
• List of Histograms.....	V
• List of Figures.....	VII
• Introduction.....	1
• Aim of the Work.....	5
• Review of literature.....	6
• Materials & Methods.....	65
• Results.....	74
• Discussion.....	126
• Summary.....	134
• Conclusion & Recommendations.....	137
• References.....	139
• Arabic Summary	

---

## *List of Abbreviations*

<b>AJCC</b>	: American joint committee of cancer
<b>APUD cells</b>	: Amine precursor uptake and decarboxylation cells.
<b>CIS</b>	: Carcinoma insitu
<b>EGFR</b>	: Epidermal growth factor receptor
<b>H-B EGF</b>	: Heparin- Binding EGF
<b>M AP K</b>	Mitogen activated protein kinase
<b>MAbs</b>	: Humanized monoclonal antibodies
<b>NSAIDs</b>	: Nonsteroidal anti inflammatory drugs
<b>PAS</b>	: Periodic acid schiff
<b>PI3-K</b>	: Phosphatidyl inositol 3 kinase
<b>RB</b>	: Retinoblastoma gene
<b>RTKs</b>	: Receptor tyrosine kinases
<b>S.</b>	: Schistosoma
<b>SCC</b>	: Squamous cell carcinoma
<b>TCC</b>	: Transitional cell carcinoma
<b>TGF <math>\alpha</math></b>	: Transforming growth factor alpha
<b>VS</b>	: Versus
<b>WHO</b>	: World health organization

## *List of Tables*

<b>Table No.</b>	<b>Title</b>	<b>Page</b>
<b>Table (1)</b>	Histologic gradation of schistosomal urinary bladder disease.	15
<b>Table (2)</b>	TNM classification of urothelial carcinoma	22
<b>Table (3)</b>	Urinary bladder staging form	27
<b>Table (4)</b>	Urinary bladder staging form	28
<b>Table (5)</b>	Histologic comparison between reactive urothelium, hyperplasia, dysplasia & CIS.	43
<b>Table (6)</b>	Sex distribution in different lesions studied.	74
<b>Table (7)</b>	Age distribution among patients studied.	76
<b>Table (8)</b>	Incidence of Bilharzial Association in different pathological lesions studied..	77
<b>Table (9)</b>	Presented Grades in different malignant lesions studied.	78
<b>Table (10)</b>	Presented Stages in Different Malignant lesions studied.	79
<b>Table (11)</b>	Relation between different grades and stages in different malignant studied lesions.	81
<b>Table (12)</b>	Effect of Bilharzial association on Tumor Grade.	82
<b>Table (13)</b>	Effect of bilharzial association on tumor stage.	83
<b>Table (14)</b>	Extent and Intensity of EGFR expression in studied cases.	84
<b>Table (15)</b>	Effect of Bilharzial association on EGFR Extent and intensity of Expression.	86
<b>Table (16)</b>	Extent and intensity of EGFR Expression in different malignant cases.	88
<b>Table (17)</b>	EGFR Expression and intensity of different studied malignant stages.	89

<b>Table No.</b>	<b>Title</b>	<b>Page</b>
<b>Table (18)</b>	EGFR Expression and Intensity of different studied malignant Stages in TCC.	90
<b>Table (19)</b>	EGFR Expression and Intensity of different studied malignant Stages in SCC.	91
<b>Table (20)</b>	Extent and intensity of EGFR expression in different studied grades.	92
<b>Table (21)</b>	Extent and Intensity of EGFR Expression in Different Studied Grades of TCC.	94
<b>Table (22)</b>	Extent and Intensity of EGFR Expression in Different Studied Grades.	96
<b>Table (23)</b>	Extent and intensity of TGF alpha expression in different studied cases.	97
<b>Table (24)</b>	Effect of Bilharzial association of TGF alpha extent and intensity of expression.	99
<b>Table (25)</b>	TGF alpha expression in different malignant studied cases.	101
<b>Table (26)</b>	Extent and intensity of TGF alpha expression in different malignant stages.	102
<b>Table (27)</b>	Extent and Intensity of TGF alpha Expression in different studied Stages in TCC.	103
<b>Table (28)</b>	Extent and Intensity of TGF alpha Expression in different studied Stages in SCC.	104
<b>Table (29)</b>	Extent and intensity of TGF alpha expression in different malignant grades.	106
<b>Table (30)</b>	Extent and Intensity of TGF alpha Expression in different studied Grades in TCC.	107
<b>Table (31)</b>	Extent and Intensity of TGFalpha Expression in different studied Grades in SCC.	109
<b>Table (32)</b>	Correlation of EGFR and TGF alpha Expression to Bilharzial Association and to Malignancy Grades and Stages.	110



## *List of Graphs*

No.	Title	Page
<b>Graph (1)</b>	The normal histology of urinary bladder	7
<b>Graph (2)</b>	Diagram shows the stages of tumor invasion in bladder cancer.	28
<b>Graph (3)</b>	Important domains of the EGFR	54
<b>Graph (4)</b>	Mechanism of action	55

## *List of Histograms*

No.	Title	Page
<b>Histograms (1)</b>	Sex distribution in different lesions studied.	75
<b>Histograms (2)</b>	Age distribution among patients studied.	76
<b>Histograms (3)</b>	Incidence of Bilharzial Association in different pathological lesions studied.	78
<b>Histograms (4)</b>	Presented Grades in different malignant lesions studied.	79
<b>Histograms (5)</b>	Presented Stages in Different Malignant lesions studied.	80
<b>Histograms (6)</b>	Relation between different grades and stages in different malignant studied lesions.	81
<b>Histograms (7)</b>	Effect of Bilharzial association on Tumor Grade.	82
<b>Histograms (8)</b>	Effect of bilharzial association on tumor stage.	83
<b>Histograms (9)</b>	Intensity of EGFR expression in positive studied cases.	85
<b>Histograms (10)</b>	Effect of Bilharzial association on EGFR intensity of Expression.	87
<b>Histograms (11)</b>	Intensity of EGFR Expression in different malignant cases.	88
<b>Histograms (12)</b>	EGFR expression intensity of different studied malignant stages.	90
<b>Histograms (13)</b>	EGFR Expression intensity of different studied malignancy Stages in TCC.	91
<b>Histograms (14)</b>	EGFR Expression intensity of different studied malignant Stages in SCC.	92

No.	Title	Page
<b>Histograms (15)</b>	Intensity of EGFR expression in different studied grades.	93
<b>Histograms (16)</b>	Intensity of EGFR Expression in Different Studied malignancy Grades of TCC.	95
<b>Histograms (17)</b>	Intensity of EGFR Expression in Different Studied Grades of SCC.	96
<b>Histograms (18)</b>	Intensity of TGF alpha expression in different studied cases.	98
<b>Histograms (19)</b>	Effect of Bilharzial association of TGF alpha intensity of expression.	100
<b>Histograms (20)</b>	TGF alpha expression in different malignant studied cases.	101
<b>Histograms (21)</b>	Intensity of Expression in different studied stages.	102
<b>Histograms (22)</b>	Intensity of TGF alpha Expression in different studied Stages in TCC.	103
<b>Histograms (23)</b>	Extent and Intensity of TGF alpha Expression in different studied Stages in SCC.	105
<b>Histograms (24)</b>	Intensity of TGF alpha expression in different malignant grades.	106
<b>Histograms (25)</b>	Intensity of TGF alpha Expression in different studied Grades in TCC.	108
<b>Histograms (26)</b>	Intensity of TGF alpha Expression in different studied Grades in SCC.	109

## *List of Figures*

No.	Title	Page
<b>Figure (1)</b>	Normal urothelium with preserved umbrella cells (Hx & Ex 200).	112
<b>Figure (2)</b>	Mild cystitis (Hx & Ex 100).	112
<b>Figure (3)</b>	Bilharzial cystitis with calcified bilharzial ova (arrows) (Hx E, x 200).	113
<b>Figure (4)</b>	Transitional cell carcinoma Grade II (Hx & E x 200).	113
<b>Figure (5)</b>	Transitional cell carcinoma, Grade III (Hx & E x 200).	114
<b>Figure (6)</b>	Well differentiated squamous cell carcinoma, (Grade I) (Hx & E x 200).	114
<b>Figure (7)</b>	Moderately differentiated squamous cell carcinoma (Grade II), (Hx & E x 200).	115
<b>Figure (8)</b>	Normal urothelium showing negative EGFR immunostaining (IHC, EGFR, DAB, x400).	115
<b>Figure (9)</b>	Bilharzial cystitis, showing mild EGFR immunoreactivity in urothelial cells, (IHC, EGFR, DAB, x 200).	116
<b>Figure (10)</b>	Chronic polypoid cystitis showing mild EGFR immunoreactivity in urothelial cells (IHC, EGFR, DAB, x 200).	116
<b>Figure (11)</b>	Transitional cell carcinoma, Grade I showing moderate EGFR immunoreactivity in malignant urothelial cells, (IHC, EGFR, DAB, x 200).	117
<b>Figure (12)</b>	Transitional cell carcinoma, Grade II showing moderate EGFR immunoreactivity in malignant cells,	117

No.	Title	Page
	(IHC, EGFR, DAB, x 400)	
<b>Figure (13)</b>	Transitional cell carcinoma, Grade II showing marked EGFR immunoreactivity in malignant cells (IHC,EGFR, DAB, x 400).	118
<b>Figure (14)</b>	Transitional cell carcinoma, Grade III showing moderate EGFR immunoreactivity in malignant cells, (IHC,EGFR,DAB x 400).	118
<b>Figure (15)</b>	Squamous cell carcinoma, Grade II showing moderate EGFR immunoreactivity in malignant cells associated with bilharziasis (IHC, EGFR, DAB, x 400).	119
<b>Figure (16)</b>	Squamous cell carcinoma, Grade III showing marked EGFR immunoreactivity in malignant urothelial cells (IHC, EGFR, DAB, x 400).	119
<b>Figure (17)</b>	Squamous cell carcinoma, Grade III showing marked EGFR immunoreactivity in malignant cells (IHC, EGFR, DAB, x 200).	120
<b>Figure (18)</b>	Normal urothelium, showing mild TGF alpha immunoreactivity in urothelial cells (IHC, TGF $\alpha$ , DAB, x 200).	120
<b>Figure (19)</b>	Chronic non- specific cystitis showing, mild TGF alpha immunoreactivity in urothelial cells (IHC, TGF $\alpha$ , DAB, x 200).	121
<b>Figure (20)</b>	Bilharzial cystitis, showing mild TGF alpha immunoreactivity in urothelial cells (IHC, TGF $\alpha$ , DAB, x 200).	121
<b>Figure (21)</b>	Papillary transitional cell carcinoma Grade II, showing marked TGF alpha immunoreactivity in malignant cells (IHC, TGF $\alpha$ , DAB, x 400).	122

No.	Title	Page
<b>Figure (22)</b>	Transitional cell carcinoma grade III, showing marked TGF alpha immunoreactivity in malignant cells (IHC, TGF $\alpha$ , DAB, x 200).	122
<b>Figure (23)</b>	Squamous cell carcinoma, Grade I associated with biharziasis (arrow), showing mild TGF alpha immunoreactivity in malignant cells (IHC, TGF $\alpha$ , DAB, x 200).	123
<b>Figure (24)</b>	Squamous cell carcinoma, Grade II associated with biharziasis, showing marked TGF alpha immunoreactivity in malignant cells (IHC, TGF $\alpha$ , DAB, x 200).	123
<b>Figure (25)</b>	Squamous cell carcinoma Grade II, showing moderate TGF alpha immunoreactivity in Grade III cells (IHC, TGF $\alpha$ , DAB, x 200)	124
<b>Figure (26)</b>	Squamous cell carcinoma, Grade II, showing marked TGF alpha immunoreactivity in malignant cells (IHC, TGF $\alpha$ , DAB, x 200).	125
<b>Figure (27)</b>	Squamous cell carcinoma associated with biharziasis, Grade II, showing mild TGF alpha immunoreactivity malignant cells (IHC, TGF $\alpha$ , DAB, x 200).	125

## Introduction

Bladder cancer is the most common malignancy involving urinary system, the fourth most incident cancer in males, and the ninth most incident in females (*Jemal et al., 2010*).

Urothelial bladder cancers have identified multiple risk factors (*Jemal et al., 2010*). In Egypt bladder cancer accounts for about 30% of all cancers, with many pathogenetic factors most commonly bilharzial infestation, which is an endemic disease in Nile river (*Ashley et al., 2008*).

Bladder cancer arises primarily from transitional cells of bladder mucosal epithelium and may be present as non invasive papillary or non papillary tumors (*Michaud et al., 2001*).

Interaction between transitional cell carcinoma cells and the adjacent or underlying bladder stroma may be an important determinant in the progression of superficial to invasive disease (*Pritchett et al., 1989*).

The Epidermal growth factor receptor (EGFR)/ human epidermal growth receptor (HER1) and its ligands epidermal growth factor (EGF) and Transforming growth factor alpha (TGF- $\alpha$ ) are important in cell proliferation, as well as motility, adhesion, invasion, survival and angiogenesis (*Gibbs, 2000*).

The EGFR is the first described member of a family of related transmembrane receptor tyrosine kinases. It is comprised of the following four related receptors: EGFR itself (ERBB1) or HER1, ERBB2 (HER2/neu), ERBB3 (HER3) and ERBB4 (HER4) (*Bekaii et al., 2006*).