Diagnostic Value of Galectin-3 as A Marker for Bladder Cancer

Thesis

Submitted For Partial Fulfillment of Master Degree

in

Clinical and Chemical Pathology

By

Sara Abd El Raxman Abd El Atty

M.B.,B.Ch., Zagazig University

Supervised By

Professor/ Xoda Moxamed El Gendi

Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Professor/ Botxina Madkour

Professor of Clinical and Chemical Pathology
Theodor Bilharz Research Institute

Doctor/ Dina Aziz Kxattab

Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine - Ain Shams University

Faculty of Medicine
Ain Shams University
2013

List of Contents

Title	Page No.

List of Abbreviations .....

List of Tables .....

List of Figures .....

List of Photos .....

Introduction .....

Aim of the work .....

Review of Literature

- **Urinary Bladder Cancer** .....
- **Bladder Cancer Biomarkers** .....
- **Galectin-3** .....

Subjects and Methods .....

Results .....

Discussion .....

Summary and Conclusion .....

Recommendations .....

References .....

Arabic Summary ..... ---

List of Abbreviations

AGE ..... : Advanced glycosylation end product
AUA ..... : American urological association
Bax ..... : Bcl-2 accelerated x protein
BCG ..... : Bacille-calmette guerin
Bcl..... : B cell lymphoma
Bcl-xL ..... : B cell lymphoma-extra-large
BH ..... : Bcl-2 homology domain
BID ..... : BH3 interacting domain death agonist
BTA..... : Bladder tumor antigen
CIS ..... : Carcinoma in situ
c-Myc ..... : Cellular-myelocytomatosis
CRD ..... : Carbohydrate recognition domain
CT..... : Computerized tomography
DNA ..... : Deoxyribonucleic acid
DR ..... : Death receptor
ECs..... : Endothelial cells
ELISA..... : Enzyme-linked immunosorbent assay
ER..... : Endoplasmic reticulum
FDPs..... : Fibrin/Fibrinogen degradation products
FISH..... : Fluorescence in situ hybridization
G I..... : Grade I
G=Gly ..... : Glycine
H&E..... : Hematoxylin and eosin.
HA ..... : Hyaluronic acid
HAase ..... : Hyaluronidase
HPV ..... : Human papillomavirus

HRP ..... : Horse radish peroxidase.
hTERT ..... : Human telomerase reverse transcriptase
hTR ..... : Human telomerase RNA component
HYAL-1..... : Hyal-uronoglucosaminidase-1
IHC ..... : Immunohistochemical.
IL ..... : Interleukin
IVP ..... : Intravenous pyelography
kDa..... : Kilo dalton.
K-Ras..... : Kirsten-rat sarcoma
LAMP..... : Lysosome-associated membrane protein
LOH..... : Loss of heterozygosity
MMPs..... : Matrix metalloproteinases
MRI..... : Magnetic resonance imaging
mRNA..... : Messenger RNA
MSA..... : Microsatellite analysis
N=Asn..... : Asparagine
NBI..... : Narrow-band imaging
NCI..... : National Cancer Institute
NMP..... : Nuclear matrix protein
p53..... : Protein 53
PBMCs..... : Peripheral blood mononuclear cells
PBS..... : Phosphate buffer saline
PCR..... : Polymerase chain reaction
PI3K..... : Phosphoinositide 3-kinase
PKB..... : Protein kinase B
PSA ..... : Prostate-specific antigen
Quanticyt ..... : Quantitative cytology.
R=Arg..... : Arginine

Raf ..... : Rapidly accelerated fibrosarcoma

RNA ..... : Ribonucleic acid

ROC ..... : Receiver operating characteristic

Rpm ..... : Revolution per minute.

RT-PCR ..... : Reverse transcriptase-polymerase chain reaction

SPSS..... : Statistical package for social sciences

SqCC..... : Squamous cell carcinoma

S-type ..... : Soluble-type

TBRI ..... : Theodor Bilharz Research Institute

TCC ..... : Transitional cell carcinoma

TCR ..... : T-cell receptor

TNM ..... : Tumor-node-metastasis

TP ..... : Telomerase-associated protein

TRAIL …… : Tumor necrosis factor (TNF)–related apoptosis-inducing ligand

TRAP..... : Telomeric repeat amplification protocol

TUR ..... : Transurethral resection

UBC ..... : Urinary bladder cancer

W=Trp..... : Tryptophan

WHO ..... : World Health Organization

WLC ..... : White light cystoscopy

Wnt ..... : Wingless/Integrated

List of Tables

Table No.	Title	Page No.
Table (1):	2009 TNM staging system.	20
Table (2):	Stages of bladder cancer.	21
Table (3):	WHO grading in 1973 and 2004	23
Table (4):	Summary of bladder cancer biomarkers and their performance.	59
Table (5):	The three main groups of the study:	84
Table (6):	Distribution of the studied groups.	112
Table (7):	Age of studied groups.	112
Table (8):	Sex distribution of studied groups.	112
Table (9):	The incidence of bilharzial association in different groups studied.	113
Table (10):	Statistical comparison between all groups as regard serum concentration of galectin-3 (pg/mL).	114
Table (11):	Comparison between SqCC and TCC as regard serum galectin-3 concentration (pg/mL).	115
Table (12):	Comparison between different grades of bladder cancer as regard serum galectin-3 concentration (pg/mL)	116
Table (13):	Comparison between different stages of bladder cancer (Group III) as regard serum galectin-3 concentration (pg/mL)	117
Table (14):	Comparison between all groups as regard galectin-3 immunohistochemical staining	118
Table (15):	Comparison between SqCC and TCC as regard galectin-3 immunohistochemical staining.	119
Table (16):	Comparison between different grades of bladder cancer as regard galectin-3 immunohistochemical staining.	120
Table (17):	Comparison between different stages of bladder cancer as regard galectin-3 immunohistochemical staining.	121

List of Tables (Cont…)

Table No.	Title	Page No.
Table (18**):**	Comparison between bilharzial and non-bilharzial cystitis as regard serum concentration of galectin-3 (pg/mL).....	122
Table (19**):**	Comparison between bilharzial and non-bilharzial cystitis as regard immunohistochemical staining of galectin-3……	121
Table (20**):**	Comparison between bilharzial and non-bilharzial associated malignant cases as regard serum galectin-3 concentration (pg/mL).....	123
Table (21**):**	Comparison between bilharzial and bon-bilharzial associated malignant cases as regard galectin-3 immuno-histochemical staining.	123
Table (22**):**	Correlation study between galectin-3 concentration (pg/mL) and galectin-3 immunohistochemistry in group III.	124
Table (23**):**	ROC curve between control and bladder cancer as regard serum concentration of galectin-3 (pg/mL).....	125
Table (24**):**	ROC curve between cystitis and bladder cancer cases as regard serum concentration of galectin-3 (pg/mL).....	127

List of Figures

Table No.	Title	Page No.
Fig. (1):	Layers of the bladder wall	6
Fig. (2):	Normal urothelium	15
Fig. (3):	Different stages of bladder cancer	19
Fig. (4):	Hexaminolevulinate fluorescence cystoscopy showing bladder tumor	29
Fig. (5):	Urine cytology stained by the Papanicolaou procedure; Normal cytology with no mitotic activity and normal nuclear- cytoplasmic ratio	42
Fig. (6):	Urine cytology showing urothelial cells with slight atypia and increased nuclear-cytoplasmic ratio	42
Fig. (7):	Urine cytology from urothelial carcinoma	43
Fig. (8):	Urovision FISH test	45
Fig. (9):	Families of galectins	62
Fig. (10):	Cellular location and functions of galectins	63
Fig. (11):	Selected biological functions assigned to different members of the galectin family	64
Fig. (12):	Extracellular functions mediated by galectin-3	69
Fig. (13):	Suggested model of the role of galectin-3 effect on Akt during apoptosis	75

List of Figures (Cont…)

Table No.	Title	Page No.
Fig. (14):	Tumor angiogenesis	78
Fig. (15):	Schematic overview of the mechanisms by which galectin expression by activated endothelial cells might contribute to tumor progression	80
Fig. (16):	Principal of ELISA technique of galectin-3.	88
Fig. (17):	Galectin-3 standard dilutions.	91
Fig. (18):	Standard curve of galectin-3 by ELISA.	94
Fig. (19):	Serum concentration of galectin-3 in different groups.	114
Fig. (20):	Serum concentration of galectin-3 in different grades of bladder cancer.	116
Fig. (21):	Serum concentration of galectin-3 in different stages of bladder cancer.	117
Fig. (22):	Galectin-3 immunostaining in different groups studied.	118
Fig. (23):	Galectin-3 immunostaining in different grades of bladder cancer.	120
Fig. (24):	Galectin-3 immunostaining in different stages of bladder cancer.	121
Fig. (25):	ROC curve between control and bladder cancer as regard serum concentration of galectin-3 (pg/mL).	125
Fig. (26):	Diagram between control and bladder cancer as regard serum concentration of galectin-3 (pg/mL).	126
Fig. (27):	ROC curve between cystitis and bladder cancer cases as regard serum concentration of galectin-3 (pg/mL).	127
Fig. (28):	Diagram between cystitis and bladder cancer cases as regard serum concentration of galectin-3 (pg/mL).	128

List of Photos

Photos No.	Title	Page No.
Photo (1**):**	Control case with normal urothelial lining (H&E x100).....	129
Photo (2**):**	Control case negative for galectin-3 monoclonal antibody (IHC, DAB x100).....	129
Photo (3**):**	Bilharzial cystitis with many dead bilharzial ova at lamina propria (H&E x100).....	130
Photo (4**):**	Bilharzial cystitis with weak expression of galectin-3 monoclonal antibody (IHC, DAB x100).	130
Photo (5**):**	Poorly differentiated squamous cell carcinoma, GIII, Stage II (H&E x200).....	131
Photo (6**):**	Poorly differentiated squamous cell carcinoma, GIII, Stage II with strong expression of galectin-3 monoclonal antibody (IHC, DAB x200).....	131
Photo (7**):**	Well differentiated squamous cell carcinoma, GI, Stage I (H&E x200).	132
Photo (8**):**	Well differentiated squamous cell carcinoma, GI, Stage I with moderate expression of galectin-3 monoclonal antibody (IHC, DAB x200).....	132
Photo (9**):**	Poorly differentiated transitional cell carcinoma, GIII, Stage II (H&E x100).....	133
Photo (10**):**	Poorly differentiated transitional cell carcinoma, GIII, Stage II with strong expression of galectin-3 monoclonal antibody (IHC, DAB x200).....	133
Photo (11**):**	Papillary non-invasive transitional cell carcinoma, GI, Ta (H&E x200).	134
Photo (12**):**	Papillary non-invasive transitional cell carcinoma, GI, Ta with moderate expression of for galectin-3 monoclonal antibody (IHC, DAB x200).....	134
Photo (13**):**	Poorly differentiated transitional cell carcinoma, GIII, Stage II associated with schistosomiasis (H&E x100).	135
Photo (14**):**	Poorly differentiated transitional cell carcinoma, GIII, Stage II associated with schistosomiasis with strong expression of galectin-3 monoclonal antibody (IHC, DAB x200).....	135

Introduction

Urinary bladder cancer is a major public health problem being one of the most common malignancies ***(Arentsen et al., 2009).*** It ranks in the top five of newly diagnosed cancers in men and in the top ten of estimated cancer deaths ***(Shelley et al., 2010).***

According to ***Blann (2006)*** the highest bladder cancer incidence rates are generally found in industrially developed countries and in areas associated with endemic schistosomiasis in Africa and Middle East. Egypt has the highest reported worldwide incidence (37 per 100,000 populations) in the world due to endemic schistosomiasis. Bladder cancer is clinically characterized by high recurrence rates and poor prognosis once the tumor invades the muscular layer ***(Jemal et al., 2009).***

Two main histological types of bladder cancer are identified; the Transitional Cell Carcinoma (TCC) and the Squamous Cell Carcinoma (SqCC). The TCC is related to cigarette smoking and is most prevalent in the Western and industrialized countries. The SqCC is more frequently seen in some Middle Eastern and African countries where urinary schistosomiasis is an endemic disease ***(Sengupta et al., 2004).***

Diagnostic procedures in bladder cancer patients include urine cytology, cystoscopy with biopsy and excretory urography.

Cystoscopy remains the standard method used for most cases of bladder carcinoma but it is an invasive procedure ***(Carmack and saloway, 2006).***

Galectin-3 is a member of the galectin gene family that is expressed at elevated levels in a variety of neoplastic cell types and has been associated with cell growth, cellular adhesion process, cell proliferation, transformation, metastasis, and apoptosis ***(Yang and Liu, 2003; Nakahara et al., 2005).***

The expression of galectin-3 is up-regulated in various types of cancer. Several reports have indicated its involvement in carcinogenesis. One possible reason for this is the anti-apoptotic activity of galectin-3. Increased galectin-3 mRNA expression compared to basal levels of normal bladder samples was observed in many bladder cancer samples ***(Takenaka et al., 2003; Takenaka et al., 2004).***

Aim of the Work

This work aims to measure serum galectin-3 concentration as well as galectin-3 expression in bladder tissues of bladder cancer patients to evaluate its role in diagnosis and to correlate these levels with different stages and grades of tumor as well as the effect of bilharziasis on its expression.

Urinary Bladder Cancer

Anatomy and Xistology:

The urinary bladder is a pelvic organ that is abdominal in position in young (< 6 years old) individuals and a pelvic organ after the pelvis has developed sufficiently, it is a hollow organ in the pelvis with flexible, muscular walls. Embryologically, it is derived from the urogenital sinus, and is initially continuous with the allantois. In males, the base of the bladder lies between the rectum and the pubic symphysis, superior to the prostate, and separated from the rectum by the rectovesical pouch. In females, the bladder sits inferior to the uterus, anterior to the vagina, and separated from the uterus by the vesicouterine pouch. In infants and young children, the urinary bladder is in the abdomen even when empty ***(Moore et al., 2006)***.

When viewed from within, the bladder is lined with *transitional epithelium*, which appears smooth when the bladder is full but contracts into numerous folds when the bladder empties. Beneath the transitional epithelium is a thin layer of connective tissue called the *lamina propria*. Next is a layer of muscle tissue called the *muscularis propria*, beyond this muscle, another zone of fatty connective tissue separates the bladder from other nearby organs ***(Fig. 1) (Kaufman et al., 2009).***