EVALUATION OF SURVIVIN AND HYALURONIDASE AS URINE MARKERS IN PATIENTS WITH BLADDER CANCER

Thesis

Submitted for Fulfillment of Master degree in Biochemistry

By

Marwa Mohanad El-Marzouki

Under Supervision of

Prof.Dr.Maged Barakat

Professor of Biochemistry

Faculty of Pharmacy

Cairo University

Prof.Dr.Sanaa Eissa

Professor of Biochemistry

Faculty of Medicine

Ain Shams University

Prof.Dr.Ashraf Saad Zaghloul

Professor of Surgical Oncology

National Cancer Institute

Cairo University

Acknowledgement

*My gratitude is for Allah**,** His merciful, for what He has given me and for all the blessings, He supplied me with, thanks **God**.*

It is a great pleasure to acknowledge the help of many indviduals without their help this work could not have been done.

*First, I really find myself lucky to be supervised by **Prof. Dr.Maged Barakat**, professor of Biochemistry, Faculty of Pharmacy, Cairo University, for his continous encouragement and sincere advice which has been the main factors to complete this work.*

*No words could express my sincere appreciation and deepest gratitude to **Prof. Dr. Sanaa Eissa**, professor of Biochemistry, Faculty of Medicine, Ain Shams University. This work was not merely a project for her, but it was a special aim to which she gave her ultimate effort and attention. Words can not find their way to express my thanks to her expertise supervision.*

*Special thanks are owed to **Prof**. **Dr.Ashraf Zagloul** Prof of Surgical Oncology, National cancer institute, Cairo University.*

Special thanks to ***Dr.Soheir Badr****, Ass.Prof.of Biochemistry, Faculty of Medicine, Ain Shams University, for her help in revising this work, May God put this work in her good deeds balance.*

I would remiss if I failed to acknowledge my parents, my sister and my friends, for their tolerance and their emotionally support that pushed me to finish this work, many thanks.

Marwa Mohanad El-Marzoki

List of contents

List of Figures

List of Tables

List of Abbreviations

AC	Adenocarcinoma
AJCC	American Joint Committee of Cancer
Ala	Alanine
ALA	Amino levulinic acid
Apaf- 1	Apoptotic Protease Activating Factor-1
b-FGF	Basic fibroblast growrh factor
BIR	Baculovirus IAP domain
BLCA-4	Bladder cancer-4
BSA	Bovine serum albumin
BTA	Bard tumor antigen
CAMs	Cell adhesion molecules
CARD	Caspase activation recuritment domain
CD34+	Bone marrow cells
CD44	Adhesion molecules
CDK	Cyclin dependant kinase
cDNA	Complementry deoxy ribonucleic acid
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in situ
CK	Cytokeratin
CT	Computed tomography
CYFRA 21-1	Cytokeratin-19
Cys	Cysteine
CYP1A2	Cytochrome P 1A2 dehydrate
DNA FCM	DNA flow cytometry
DNA	Deoxy ribonucleic acid
EC	Endothelial cell
ECM	Extracellular matrix
ELISA	Enzyme linked immunosorbent assay
FDP	Fibronogen degradation producr
FGF	Fibroblast growth factor
FISH	Fluorescence in-situ hybridization

GPI	Glycosyl phosphatidyl inositol
HA	Hyaluronic acid
HAase	Hyaluronidase
hALA	Ester derivative of ALA
HBXIP	Hepatitis B interacting protein
HSP90	Heat shock protein-90
hTERT	Human telomerase reverse transcriptase
Hyal	Hyaluronidase enzyme
HYAL	Hyaluronidase gene
IAP	Inhibitor of apoptosis
ICE	Interleukin -1ß-Converting Enzyme
IHA	Indirect haemagglutination test
ISUP	International Society of Urological Pathology
IVP	Intravenous pyelogram
MMP	Matrix metalloproteinase
MRI	Magnetic resonanace imaging
mt-DNA	Mitochondrial DNA
NAT1	N-acetyl transferase 1
NAT2	N-acetyl transferase 2
NCI	National Cancer Institute
NF	Nuclear factor
NMP22	Nuclear matrix protein
NNA	N-nitrosamines
NPV	Negative predictive value
p53	Protein 53 (oncogene product)
PA	Plasminogen activator
PAH	Polycylic aromatic hydrocarbons
PARs	Plasminogen activator receptors
PCR	Polymerase chain reaction
Pg	Picogram
PI3K	Phosphoinositol-3 kinase
PPV	Positive predictive value
PIN	Prostatic intraepithelial neoplasia

pro-IL-18	Proinflammatory cytokines
pro-IL-1ß	Proinflammatory cytokines
PT	Permeability transition
RASSFIA	Tumor suppressor gene
Rb	Retinoblastoma protein
RBCs	Red blood cells
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcriptase polymerase chain reaction
RZ	Ribozyme
siRNA	Small interfering RNA
SCC	Squamous Cell Carcinoma
SD	Standard of deviation
STAT-3	Signal transducer and activator of transcription3
TBS	Tris buffer saline
TCC	Transitional Cell Carcinoma
Thr	Threonine
TNF	Tumor necrotic factor
TRAP	Telomeric repeat action protocol
UBC	Urinary bladder cancer test
VDAC	Voltage dependent anion channel
VEGF	Vascular endothelial growth factor
WHO	World health organization

Abstract

Purpose: To evaluate a convenient and non invasive procedure to diagnose bladder cancer by examining the usefulness of urinary survivin and HAase RNA in diagnosis of bladder cancer and to evaluate their sensitivity and specificity in comparison to urine cytology. Also, the study correlates these factors with different clinicopathological factors.

Materials and Methods: The study was done on 100 urine cases; 60 cases were collected from malignant bladder cancer patients; 20 cases were collected from benign bladder patients and 20 cases were collected from apparently normal individuals. Two factors survivin “antiapoptotic protein”and hyaluronidase were detected in urine specimens of the different studied groups. Survivin was quantitatively measured by using enzyme linked immunosorbent assay (ELISA). HAase was detected in urine pellets by RT-PCR.

Results: Survivin and HAase were significantly increased in malignant group than benign or normal control group. There was no significant relationship between survivin and HAase with different clinicopathological factors. The sensitivity of HAase alone was 86.67% while that survivin of and urine cytology was 78.33% and 38.33%, respectively. The sensitivity of urine cytology was increased on combination with either survivin or HAase. Also, combination of both markers increased overall sensitivity.

Conclusion: Inspite of slightly lower sensitivity of survivin 78.33% than HAase 86.67%, survivin detection has the advantage of being a quantitative test measured by ELISA which is of lower cost and easily performed than RT-PCR. Many precautions should also be considered on collection, transport and storage of samples when detecting HAase RNA on these samples. Combined use of cytology with survivin and HAase was the best recommended combination for bladder cancer detection.

KEY WORDS: Bladder cancer; apoptosis; survivin; hyaluronidase; cytology

INTRODUCTION

Bladder cancer represents a significant public health problem leading to more than 130,000 worldwide deaths annually. Disease prevalence is remarkable where more than 500,000 patients carrying the disease in United States alone **(Borden *et al.*, 2003).**

Carcinoma of the bladder is the most prevalent cancer in Egypt and in most African countries. At National Cancer Institute (NCI), Cairo, it constitutes 30.3% of all cancers **(El-Mawla *et al.*, 2001).**

Early detection of high grade carcinoma may help to improve the prognosis of these cases. Cystoscopy along with cytology is the main tool for diagnosis of bladder cancer. Cytology is specific for diagnosis of bladder carcinoma but less sensitive particularly in low-grade disease. Cystoscopy on the other hand is invasive but relatively costly technique and may be inconclusive at times particularly in case of cystitis. These characteristics have prompted the search for more reliable noninvasive markers of bladder cancers **(Ianari *et al.*, 1997).**

A noninvasive method for the detection of urothelial carcinomas of urinary bladder would help improve assessment and follow up of patients with bladder carcinoma, as well as improve screening of high-risk groups, such as patients with schistomiasis and smokers, for the development of these malignancies **(Eissa *et al.*, 2004).**

Regulation of cell proliferation by programmed cell death (apoptosis) contributes to tissue and organ homeostasis during development and differentiation. This process involves an evolutionary conserved multistep cascade and is controlled by proteins that promote or counteract apoptotic cell death **(Adida *et al.*, 1998b).**

Increasing resistance to programmed cell death by an imbalance between proapoptotic protein and anti-apoptotic protein plays a critical role during tumorigenesis and tumor progression facilitating the accumulation of transforming mutation and promoting evasion of tumor cells from immunosurveillence. A number of gene products with anti-apoptotic potential are known to modulate tumor cell viability and