

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

***Thesis***

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## **List of Abbreviations**

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

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

<b>pro-IL-18</b>	Proinflammatory cytokines
<b>pro-IL-1<math>\beta</math></b>	Proinflammatory cytokines
<b>PT</b>	Permeability transition
<b>RASSF1A</b>	Tumor suppressor gene
<b>Rb</b>	Retinoblastoma protein
<b>RBCs</b>	Red blood cells
<b>RNA</b>	Ribonucleic acid
<b>ROS</b>	Reactive oxygen species
<b>RT-PCR</b>	Reverse transcriptase polymerase chain reaction
<b>RZ</b>	Ribozyme
<b>siRNA</b>	Small interfering RNA
<b>SCC</b>	Squamous Cell Carcinoma
<b>SD</b>	Standard of deviation
<b>STAT-3</b>	Signal transducer and activator of transcription3
<b>TBS</b>	Tris buffer saline
<b>TCC</b>	Transitional Cell Carcinoma
<b>Thr</b>	Threonine
<b>TNF</b>	Tumor necrotic factor
<b>TRAP</b>	Telomeric repeat action protocol
<b>UBC</b>	Urinary bladder cancer test
<b>VDAC</b>	Voltage dependent anion channel
<b>VEGF</b>	Vascular endothelial growth factor
<b>WHO</b>	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:** In spite 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 immunosurveillance. A number of gene products with anti-apoptotic potential are known to modulate tumor cell viability and