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*Diagnostic Evaluation of Urinary Survivin and
Matrix Metalloproteinases in Patients with
Bladder Cancer*

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ABSTRACT

Urinary tumor markers that help in the early detection of bladder cancer promise a significant improvement in sensitivity, specificity and convenience over conventional, invasive diagnostic tests. Regulation of cell proliferation by programmed cell death (apoptosis) contributes to tissue and organ homeostasis during development and differentiation. Abnormalities in the control of programmed cell death have an important role in tumorigenesis. Survivin, also called Baculoviral IAP repeat-containing 5 (BIRC5), is a human gene that is part of the inhibitor of apoptosis family (IAP). The survivin protein functions to inhibit caspase activation therefore leading to negative regulation of apoptosis or programmed cell death. The MMPs (matrix metalloproteinase) are a family of zinc-dependent endopeptidases that have been associated with the ability of tumor cells to degrade extracellular matrix components during tumor cell invasion

We assessed the diagnostic efficacy of Survivin, MMPs (matrix metalloproteinases) for early bladder cancer detection. The study included 46 patients diagnosed with bladder carcinoma, 20 with benign bladder lesions and 20 healthy volunteers who served as controls. All underwent serological assessment of schistosomiasis antibodies, urine cytology, and survivin RNA determination by qualitative reverse transcriptase-polymerase chain reaction and Detection of

MMP-2, MMP-9 by gelatin zymography in urothelial cells from voided urine.

Th result show positivity rates of survivin RNA on qualitative reverse transcriptase-polymerase chain reaction and MMPs zymography were significantly different among the 3 groups. The urine survivin detection by qualitative RT Nested PCR as a tumor marker showed 76% sensitivity, 95% specificity The overall sensitivity, specificity of urinary MMP zymography was 67.3%, 90%. The combined use of urine cytology and different investigated parameters such as qualitative RT Nested PCR of urine survivin and urinary MMPs zymography, increased sensitivity of urine cytology from 50% to 84.7% and 84.7% respectively with decrease in its specificity from 100% to 95% and 90% respectively.

The detection of urinary survivin RNA and MMPS zymography are a promising noninvasive test for bladder cancer early detection. Survivin quantitative reverse transcriptase-polymerase chain reaction and MMPs zymography are favored for its high sensitivity and specificity. furthermore, combined testing of cytology with them improves the sensitivity even in superficial and low-grade tumors.

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LIST OF ABBREVIATIONS

AJCC	: American Joint Committee of cancer.
APC	:Adenomatous polyposis coli
ASP 71	:Aspartic acid at position 71.
ALL	: Acute lymphocytic leukemia
AML	: Acute myelocytic leukemia
Ang-1	: Angiopoietin-1
AS-ODN	: antisense oligodeoxynucleotides
AURK	: Aurora kinase
APAF1	: apoptotic- protease-activating factor-1
α1PI	: α 1-proteinase inhibitor
α2M;	: α 2-macroglobulin
β	: Beta
BAEC	: Bovine aortic endothelial cells
Bak	: Bcl-2 antagonist/killer.
Bax	:Bcl-2 associated x protein
bp	: Base pair.
BC	: Bladder cancer.
BCC	: Basal cell carcinoma
Bcl-2	:B-cell lymphoma 2. Belongs to the Bcl-2 family of proteins and is known to inhibit apoptosis.
Bcl-xL	:Long form of Bcl-x.
Bcl-w	:A member of the Bcl-2 family that promotes cell survival.
Bid	:BH-3 Interacting Domain Death agonist that induces ICE-like proteases and apoptosis.
Bim	: A member of the Bcl-2 family that promotes apoptosis.
BIR	:A baculovirus IAP repeat motif .
BIRPs	: BIR-domain containing proteins

BLCA	: Novel nuclear matrix protein.
BPH	: Benign prostate hyperplasia.
BTA	: Bard tumour Antigen test.
BSA	: Bovine serum albumin.
CARD	: Caspase-recruiting domain
C	: Cysteine
CA	: Cysteine Array
cDNA	: Complementary deoxy ribonucleic acid.
CD44	: Cluster of differentiation number 44.
CDKs	: Cyclin dependent kinases.
CDKN2	: Cyclin dependent kinases-4 inhibitor of p16.
CEA	: Carcinoembryonic antigen.
CFH	: Complement factor H.
CFH-rp	: Complement factor H related protein.
Ch	: Chicken
Cip family	: Cyclin dependent kinase inhibitory protein.
CIS	: Carcinoma in situ.
cIAP1	: Cellular inhibitor of apoptosis protein 1.
cIAP2	: Cellular inhibitor of apoptosis protein 2.
CK	: Cytokeratin.
CLL	: Chronic lymphocytic leukemia
CML	: Chronic myelocytic leukemia
CNS	: Central nervous system
CPG	: cytosine base linked to guanine by phosphodiester bond.
CPC	: chromosomal passenger complex
CRM1	: chromosome region maintenance protein 1
CTL	: cytolytic T cells
CT	: Computerized Tomography.
CYFRA	: Cytokeratin fragments.
CYP₁A₂	: Cytochrome type P ₁ , subtype A ₂ .

D310	: Mononucleotide repeats in the mitochondrial DNA.
dbEST	: Expressed sequence tags database
DD	: Death domain.
DEPC	: Diethyl pyro-carbonate.
DIAP	: Drosophila inhibitor of apoptosis
DIABLO	: Direct IAp Binding protein with LOw pI (Diablo) .
dNTP	: Deoxy nucleotide triphosphate.
E2F	: Elongation factor 2.
EC	: Endothelial cells.
ECM	: extracellular matrix
E-cadherin	: Epithelial type cadherin.
EDTA	: Ethylenediamine tetra-acetic acid.
EGF	: Epidermal growth factor.
EGFR	: Epidermal growth factor receptor.
ELISA	: Enzyme linked immunosorbant assay.
EPR-1	: Effector cell protease receptor-1
Erb- B2	: Erythroblastic leukemia oncogene homology-2
EST	: Expressed sequence tags
Fas	: Fibroblast associated factor
FDA	: Food and Drug Administration.
FDPs	: Fibrinogen degradation products.
FGF	: Fibroblast growth factor.
FGFR	: Fibroblast growth factor receptor.
Fig.	: Figure.
FISH	: Fluorescence in situ hybridization.
G 1	: Growth phase 1.
GC content	: Guanine cytosine content.
GF	: Growth factor
GPI	: Glycosylphosphatidylinositol

HA	:Hyalouronic acid.
HBXIP	:hepatitis B X-interacting protein
HER2/neu	: (also known as ErbB-2).
HPV	:Human papiloma virus.
HRAS	:Harvey rat sarcoma virus oncogene.
HSP90	: heat shock protein 90
HSILs	:High grade squamous intraepithelial leision.
HUVECs	: Human umbilical vein endothelial cells
hTERT	: Human telomerase reverse transcriptase.
hTR	: Human telomerase RNA.
IHA	: Indirect haemagglutination.
IAPs	:Inhibitor of apoptosis proteins.
IHC	: Immunohistochemistry technique.
IL	:Interleukin.
INCEP	;Inner centromer protein.
INK4 family	: Inhibitors of cdk4 .
IPMT	: Intraductal papillary-mucinous tumor
ISUP	:International society of urological pathology.
I.V.P	: Intravenous pyelography.
KCL	: Potassium chloride.
kDa	:Kelo Dalton
KIP family	: Kinase inhibitory protein family.
LOH	: Loss of heterozygosity.
LSILs	: Low grade squamous intraepithelial leision.
(M)	: Metastasis.
M	: Molar.
MAPK	: Mitogen activated protein kinase.
MBOCA	:4,4` methylene bis (2-choloroaniline).
MBP	: myelin basic protein,
MCAK	: mitotic centromere-associated kinesin
Mdm2	: murine double minute oncogene.
MEN	:mitotic exit network

MEK	:Map/Erk kinase .
MgCL₂	: Magnesium chloride.
MIAP	:Mouse inhibitor of apoptosis protein. MIAP is a homologue to human XIAP .
MMP	: Matrix metalloproteinase.
M-MuLV	: Moloney Murine leukemia virus.
Mn⁺⁺	: Manganese.
MRI	: Magnetic Resonance Imaging.
mRNA	: Messenger ribonucleic acid.
mtDNA	: Mitochondrial Deoxy nucleic acid.
mTOR	: Mammalian target of rapamycin
MVD	:Microvascular density.
MVECs	:Microvascular endothelial cells
MS	: Multiple sclerosis
m-survivin	: Murine survivin
Myc-oncogene	: Myc is a nuclear protein that belongs to the helix-loop-helix / leucin zipper family of transcriptional factors.
MW	:molecular weight
N	: Lymph node.
n	: Number.
N/A	:Not applicable
NAIP	: Neuronal apoptosis inhibitory protein. NAIP is a member of the IAP family and inhibits apoptosis.
NAT₁	: N acetyl transferase 1.
NAT₁*₁₀	: N acetyl transferase 1 allele type 10.
NAT₂	: N- acetyl transferase 2.
NCI	: National Cancer Institute.
ND	: Not determined
NED	:No evidence of disease.
ng	: Nanogram.

NHL	: Non-Hodgkin's lymphoma
NK	: Natural killer
nm	: Nanometer.
NMR	: Nuclear magnetic resonance
NMP22	: Nuclear m atrix p rotein 22.
NPV	: Negative predictive value.
NS	: Non significant.
NSAIDs	: Non steroidal anti inflammatory drugs.
NSA-BC	:Non shistosoma associated bladder cancer.
NSCLC	: Non-small-cell lung cancer
p53	: Protein of molecular weight 53.
p16^{INK4a}	:is a tumor-suppressor which inhibits the cyclin dependent kinases 4 and 6.
p21 WAF1/CIP1	:encodes a 21 kDa protein ,p21 was simultaneously identified as WAF1 (Wild-type p53-Activated Fragment1) and as CIP1 (Cyclin-dependent kinase Inhibitor Protein).
P34cdc	:Serine theronine protein kinase wighted 34Kd.
Pad-T43A	:Replication deficient adenovirus encoding survivin Theronine→Alanine.
PAHs	: Polycyclic aromatic hydrocarbons.
PAGE	: polyacrylamide gel electrophoresis.
PBS	: Phosphate buffer saline.
PCAH	: Polycyclic aromatic hydrocarbon.
PCD	:Programmed cell death.
PCR	: Polymerase chain reaction.
Pd (N)₆	: Poly-deoxy nucleotide-6.
PDGF	: Platelet derived growth factor.
PDC	: Pancreatic ductal cell adenocarcinoma
PEL	:Primary effusion lymphoma.
PET	:Positron Emission Tomography.

pg	: Picogram.
PKA	:Protein kinase A
PKB	:Protein kinase B (heat inducing apoptosis protein) .
PKC	:Protein kinase C
Pmol	: Picomole.
PMSF	: Phospho-methyle-sulphonyl fluoride.
PPV	: Positive predictive value.
PP2A	:protein phosphatase 2A
pRB	: Protein product of the retinoblastoma gene.
PSA	: Prostatic cancer antigen.
PT3-K	:phosphotidyl inositol 3 kinase.
rRNA	: ribosomal RNA.
RASSFIA	:RAS associated family
RBcs	: Red blood cells.
RCC	: Renal cell carcinoma
RCA	: Regulator of complement activation.
RGD	: Arg-Gly-Asp sequence.
RNA	: Ribonucleic acid.
RT-PCR	:Reverse transcriptase polymerase chain reaction.
S	: Significant.
SA-BC	: Schistosoma-associated bladder carcinoma
SCC	: Squamous cell carcinoma.
SCID	:Severe combined immunodeficient mice
SGO2	: shugoshin 2
S phase	: Synthetic phase of the cell cycle.
sFAS	:Soluble FAS.
SD	: Standard deviation.
Sdi	:scenencse cell derived inhibitor of DNA synthesis.
SDS	: Sodium dodecyl sulfate.
Smac	:Second Mitochondria-derived Activator of Caspases

SMAC DIABLO	:direct inhibitor of apoptosis binding protein with low pH
STAT	:Signal Transducer and Activator of Transcription.
SPSS	: Statistical Package for the Social Sciences software.
Taq DNA	: Thermus aquatica deoxy ribonucleic acid.
TBE buffer	: Trisma-Boric-EDTA-buffer.
TCC	: Transitional cell carcinoma.
TCF4	:Transcription factor 4
TFα	: Tumour factor α .
TGF-β	: Transforming growth factor β .
Thr 34	:Theronine at 34 position.
TIMPs	: Tissue inhibitors of metalloproteinases
Tm	: Melting temperature.
TNF-α	: Tumor necrosis factor α .
TNFR	: Tumor necrosis factor receptor.
TPA	:Tissue polypeptide antigen test.
TPS	:Tissue polypeptide specific test.
TNM	: Tumor-nodes-metastases.
TRAP	: Telomeric repeat amplification protocol.
Tris-HCL	:Tris (hydroxymethyl) amino methane hydrochloride.
TSC1	:Tuberous sclerosis 1 gene.
TSG	: Tumour suppressor gene.
TSNAs	:Tobaco specific nitrosamines.
Tth	: Thermus thermophilus.
TURT	: transurethral resection of tumour.
UBC test	: Urinary bladder cancer test.
Ucs	:Uretherocystescopy.
UCD	: Ubiquitin-conjugating domain

UFD1	:ubiquitin fusion degradation - 1
UTI	:Urinary tract infection.
UVB	: Ultraviolet beam.
VUC	:Voided urine cytology.
VEGF	: Vascular endothelial growth factor.
WAF	:Wild non mutated p53.
WBcs	: White blood cells.
WB	:Western blot
WHO	: World health organization.
Wt-p53	:Wild type P53.
Xg	: Times gravity.
X-IAP	: X chromosome-linked inhibitor of apoptosis
XIAP	:X-linked inhibitor of apoptosis protein. XIAP, also known as HILP.
μmol	: Micromole.
μg	: Microgram.
μL	: Microlitre.
μM	: Micromolar.
9p	: Segment 9 of the short arm of chromosome.
21q	: Segment 21 of the long arm of chromosome.