INTRODUCTION

Bladder cancer is one of the most common urologic malignancies which accounts for 3% of all cancer-related death, especially in Egypt where schistosoma is endemic. Of affected patients, 50% to 80% experience local tumor recurrence with tendency to progression in 10% to 20% of all cases ***(Jemal et al., 2005)***.

Bladder cancer is a disease with variable clinical behavior which shows early relapse ***(Ozen and Hall, 2000)***. Cystoscopic visualization with biopsy remains the standard procedure for its detection and surveillance for recurrence. Due to the need for frequent follow-up, the invasive nature of cystoscopy and the lack of sensitivity and specificity of current screening procedures, there is a critical need for the identification of new non-invasive diagnostic markers ***(Moussa et al., 2006)***.

Apoptosis has a critical role in normal morphogenesis and homeostatic mechanisms. The suppression of apoptosis by aberrantly prolonging cell viability contributes to carcinogenesis and cancer progression ***(Thompson, 1995)***. Survivin, a human member of the inhibitors of apoptosis protein family, is the most effective one which negatively regulates cell death. Survivin is a 16.5 kDa cytoplasmic protein, its chromosomal location is at 17q25, 3% of the distance from telomere. Survivin interferes with mitosis by directly inhibiting caspase-3 and caspase-7 or conjugate caspase-9 and regulates

the G2/M phase of the cell cycle by interacting with spindle microtubules ***(Giodini et al., 2002)***.

Smith et al. (2001) found that survivin is overexpressed in bladder cancer tissues and its concentration is positively correlated with the stage of the disease. Several studies proved that reverse transcriptase-polymerase chain reaction (RT-PCR) is a sensitive method that can detect messenger ribosomal nucleic acid (mRNA) of genes in a minute amount of cells. Therefore, detection of survivin mRNA in urine sediments using RT-PCR, may provide a sensitive diagnostic tool for the detection and follow-up surveillance protocol of bladder cancer patients ***(Moussa et al., 2006)***.

AIM OF THE WORK

The aim of this study is to evaluate the potential clinical utility of survivin mRNA in urine specimens as a non-invasive marker for bladder cancer detection and monitoring as regards sensitivity and specificity. Also urinary survivin mRNA will be correlated with clinicopathologic characteristics of patients including pathological stage and tumor grade.

I. BLADDER CANCER

A. Epidemiology of Bladder Cancer:

Bladder cancer ranks fourth in incidence among all cancers in males and ninth in females worldwide. Annually, more than 54,000 new cases are diagnosed in the United States and nearly 12,000 deaths are attributed to this disease. Males have fourfold excess of bladder cancer compared with females across all racial-ethnic groups ***(Alcaraz, 2007)***.

Despite the marked decrease in prevalence of endemic bilharziasis over the last two decades, Egypt is still paying the toll of the previously high prevalence of the disease. Bladder cancer is the most common cancer in Egypt (10.1% of all cancers). It ranks first among all types of cancer reported in males (16.2% of male cancers), and has a male preponderance of 5 to 1 ***(Khaled et al., 2005).***

Worldwide, the disease is rare prior to age of 35, and two-thirds of cases occur in people aged 65 or older ***(Ross et al., 2006)***. In Schistosoma-related bladder cancer (e.g., in Egypt), its incidence reaches a peak between the third and fifth decades of life ***(Abd El Gawad et al., 2005).***

B. Risk Factors:

1. Cigarette Smoking:

Cigarette smoking is the most important risk factor for bladder cancer worldwide. It increases the risk threefold

compared to lifelong non-smokers. Although infection with schistosomiasis is linked to development of bladder cancer throughout Africa and parts of Asia, in Western countries cigarette smoking is the most closely linked risk factor, contributing to >50% of cases of bladder cancer. This is related to the presence of carcinogenic arylamines. The term "aryl" refers to any functional group derived from a simple aromatic ring, e.g., phenyl derived from benzene, xylyl from xylene and tolyl from toluene. The main arylamines present in cigarettes include 2-naphthylamine and 4-aminobiphenyl ***(Castelao et al., 2001 and Kirkali et al., 2005)***. Risk increases with increasing number of cigarettes smoked on a daily basis and with duration of smoking, and is higher in females for a given dose and duration. The risk among ex-smokers is intermediate between current and non-smokers. Pipe and cigar smoking are weakly related to bladder cancer risk. Further studies are required on the impact of environmental smoke exposure (passive smoking) on bladder cancer development ***(Ross et al., 2006).***

2. Occupational Exposure:

Various industries are linked to bladder cancer. The most important is the synthetic dye industry, especially the arylamine "aniline" dye, which increases the risk 20-fold. Occupations at high risk include those of the textile fabric, leather industry, commercial painters, hair dressers (exposed to arylamines in hair dyes), rubber workers, truck drivers and aluminum workers. All these are related to exposure to arylamines ***(Kirkali et al., 2005).***

3. Persistent Urinary Tract Infection:

Chronic inflammation increases the risk of bladder cancer. A typical example is chronic infestation with Schistosoma haematobium. In Egypt, schistosoma haematobium control programs have lowered the prevalence of the infection from 35% in 1983 to 1.7% in 2003. Bladder carcinogenesis is probably related to bacterial and viral infections commonly associated with bilharzial infestation rather than the parasite itself ***(Mostafa et al., 1999)***. Recently, ***Messing (2007)*** reported that bacteria are fixed on the cutaneous surface of the worms in clearly defined places and colonize the cecum of the parasite. He also recorded that human papilloma virus is detected in up to 30 % of cases of bilharzial bladder.

Heavy egg deposits in the bladder mucosa and submucosa can act as a mechanical irritant to the urothelium. This induces chronic inflammatory lesions, with subsequent hyperplasia and squamous metaplasia. Furthermore, vitamin A deficiency, which is common in Egyptian farmers, also contributes to the high frequency of squamous metaplasia of bladder epithelium and hence the predominance of squamous cell carcinoma (SCC) in bilharzial patients. The latter constitutes about 60%-90%, meanwhile 5%-15% are adenocarcinoma and a small proportion is transitional cell carcinoma ***(Shokeir, 2004).***

Another example of predisposing urinary tract infection is chronic cystitis in the presence of indwelling catheters or

calculi. This is associated with an increased risk for SCC of the bladder. In this respect, it has been reported that between 2% and 10% of paraplegics with long-term indwelling catheters develop bladder cancer, 80% of which are SCC ***(Hamid et al., 2003).***

4. Analgesic Abuse:

Consumption of large quantities, cumulatively 5 to 15 kg over a 10-year period, of analgesic combinations containing phenacetin is associated with an increased risk for transitional cell carcinoma (TCC) of the renal pelvis and bladder. This is because phenacetin has a chemical structure similar to that of aniline dyes ***(Messing, 2007).***

5. Dietary Factors:

Two artificial sweeteners, cyclamate and saccharin, were found to induce bladder tumors, both independently and as enhancers of the effects of other carcinogens. Fortunately, this does not occur in doses that humans routinely consume ***(Dybing, 2002)***.

Studies on caffeine-containing beverages are inconsistent. Those revealing an increased risk suggest only a modest increase, probably at the limit of detectability by epidemiological methods ***(Yang et al., 2007)***.

Some food, including preserved meat such as hot dogs and salami, provide a source of nitrosamines, which are chemicals predisposing to bladder cancer. These can also be

formed in vivo from ingested nitrates and secondary amines by nitrate-reducing bacteria in the human bladder ***(Garcia-Closas et al., 2007).***

C. Pathogenesis:

1. Arylamine Metabolism:

The arylamines present in cigarette smoke or in the workplace require metabolic activation to transform into their carcinogenic reactive metabolites. Hepatic N-hydroxylation is a critical first step. There is evidence that bioactivation also takes place in extrahepatic tissues, catalyzed by cyclo-oxygenases 1 and 2. The hydroxylamines can form adducts with hemoglobin, or can circulate as free or glucuronidated compounds eventually excreted through the kidney. These latter compounds are hydrolyzed in the acidic environment of the bladder lumen and, with or without further local bioactivation by N-acetyl-transferase 1, can covalently bind to urothelial deoxyribonucleic acid (DNA). Misrepair of the damage to DNA induced by these adducts can lead to mutations in proto-oncogenes and/or tumor-suppressor genes, a critical step in the process of transforming a normal cell to its malignant phenotype ***(Wiese et al., 2001)***.

Glutathione-S-transferase M1 (GSTM1) is part of a family of cytosolic glutathione transferase enzymes. These are dimeric enzymes with subunits of 199 - 244 amino acids in length that catalyse conjugation of electrophilic substrates with glutathione resulting in detoxification of reactive chemical entities, including arylamine metabolites. GSTM1 is

polymorphic in humans: GSTM1-null individuals experience a 30 to 50% increase in risk for bladder cancer compared to those with one or two copies of the gene ***(Engel et al., 2002 and Hayes et al., 2005)***.

2. Chronic Inflammation:

Chronic inflammation provides a promoting factor for increasing the rate of cell turnover via induction of restorative hyperplasia and squamous metaplasia. The proliferating cells are at first not neoplastic but transitional and non-invasive. Most of these focal hyperplasias are subsequently reversible. However, in some situations, hyperplasia and dysplasia may become irreversible, particularly during concomitant exposure to low doses of carcinogens, for example, N-nitroso compounds which are present in bacon, tobacco smoke and some cosmetics ***(Mostafa et al., 1999 and Mirvish et al., 2002)***. Urinary bacteria also play a role by a double action: the secretion of β-glucuronidase enzyme which cleaves conjugated carcinogens yielding free carcinogenic products, and the production of carcinogenic nitrosamines from their precursors in urine, e.g., nitrates and secondary amines ***(Shokeir, 2004).***

3. Molecular Pathogenesis:

Two main categories of genes are implicated in malignant transformation: oncogenes and tumor suppressor genes. Transformation of a proto-oncogene into an oncogene results in its overexpression. This transformation may be caused

by a mutation, gene amplification, promoter methylation or insertion of viral genetic material into the human DNA. Activation of the oncogene results in derangement of cell cycle control, thereby stimulating malignant transformation ***(Knowles, 2001)***.

Tumor suppressor genes have two alleles. If only one allele for the gene is damaged, the second can still produce the correct protein. Therefore, both must be inactivated in order to induce tumorigenesis. The proposed mechanisms for this event are via loss of, or mutation in, both alleles or loss of heterozygosity in one and mutation in the sequence of the remaining allele ***(Nakagawa et al., 2003).***

Alterations in chromosomes 9 and 17 play important roles in the development and progression of bladder cancer. The tumor suppressor genes on chromosome 9 have been implicated, marked by allelic losses seen in both 9p and 9q. Allelic loss only on chromosome 9 is found exclusively in low-grade superficial tumors. These have a good prognosis and a low propensity to invade and metastasize. In contrast, loss of genetic material on chromosome 17p, the location of p53 tumor suppressor gene, is associated with high grade tumors demonstrating a more aggressive behavior. This indicates the existence of at least two divergent pathways of bladder tumor progression that can be defined by unique patterns of molecular alterations ***(Cote et al., 2006)***.

D. Protective Factors:

Non-steroidal anti-inflammatory drugs are recognized chemopreventive agents due to their inhibitory actions on the expression of cyclo-oxygenase 2. Epidemiologic studies demonstrated a lower risk of bladder cancer for regular and sustained users of non-steroidal anti-inflammatory drugs ***(Castelao et al., 2000)***.

Some dietary antioxidants prevent carcinogen-induced bladder cancer. Smokers ingesting high levels of carotenoids have lower hemoglobin adduct levels of 3- and 4-amino-biphenyl (carcinogenic arylamines) than those with lower intake of this dietary antioxidant. Similarly, smokers consuming high levels of vitamin C have low levels of arylamine hemoglobin adducts. In addition, vitamin C blocks in-vivo nitrosamine formation ***(Castelao et al., 2004).***

Total fluid intake also appears to be associated with reduced risk of bladder cancer. In a 10-year study involving almost 48,000 men, researchers found that men who drank 1.5 L of water a day had a significantly reduced incidence of bladder cancer when compared with men who drank less than 240 mL per day ***(Ross et al., 2006)***. The authors proposed that bladder cancer might partly be caused by the bladder directly contacting carcinogens that are excreted in urine. It was postulated, therefore, that by drinking higher quantities of water, urine is more dilute, thereby diluting carcinogens in urine and/or by reducing contact time between urine carcinogens and bladder epithelium due to increasing frequency of micturition, so reducing the chance of the disease.

E. Pathology:

The urinary bladder is lined by a multilayered epithelium known as transitional epithelium or urothelium. It is composed of a basal cell layer on which rests several layers of intermediate cells, with small round nuclei and small amount of cytoplasm. The superficial layer is composed of larger cells referred to as "umbrella" cells having abundant cytoplasm and more than one nucleus ***(Young and Mckee, 2006).***

1. Histopathological Grading of Bladder Cancer:

No uniformly accepted grading system for bladder cancer exists. The most commonly used system is based on the degree of cellular anaplasia and includes:

- Grade 1: well-differentiated tumors.
- Grade 2: moderately differentiated tumors.
- Grade 3: poorly differentiated tumors.

A strong correlation exists between tumor grade and stage, with most well- and moderately differentiated tumors being superficial and most poorly differentiated ones being muscle invasive ***(Messing, 2007).***

2. Histopathological Staging of Bladder Cancer:

Bladder tumors are staged according to the American Joint Committee on Cancer (AJCC) Tumor-Node-Metastasis (TNM) system, shown in *Tables 1 and 2*, and illustrated in *Figure 1* ***(Koppie and Bochner, 2006).***

Table (1): Tumor-Node-Metastasis (TNM) Staging System

Primary tumor (T)	Tx	Primary cannot be assessed
	T0	No evidence of primary tumor
	Ta	Noninvasive papillary tumor
	Tis	Carcinoma *in situ*
	T1	Tumor invades subepithelial connective tissue
	T2	Tumor invades muscle
	T2a	Tumor invades superficial muscle (inner half)
	T2b	Tumor invades deep muscle (outer half)
	T3	Tumor invades perivesical tissue
	T3a	Tumor invades perivesical tissue microscopically
	T3b	Tumor invades perivesical tissue macroscopically (extravesical mass)
	T4	Tumor invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall
	T4a	Tumor invades prostate, uterus, vagina
	T4b	Tumor invades pelvic wall, abdominal wall
Regional lymph nodes (N)	NX	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastases
	N1	Metastasis in a single lymph node, 2 cm or less in greatest dimension
	N2	Metastasis in a single lymph node, more than 2 cm but not more than 5 cm in greatest dimension; or multiple lymph nodes, none more than 5 cm in greatest dimension
	N3	Metastasis in a lymph node, more than 5 cm in greatest dimension
Distant metastasis (M)	MX	Distant metastasis cannot be assessed
	M0	No distant metastases
	M1	Distant metastasis

(Koppie and Bochner, 2006)

Table (2): American Joint Committee on Cancer Stage Groupings

Stage 0a	Ta, N0, M0
Stage 0is	Tis, N0, M0
Stage I	T1, N0, M0
Stage II	T2a, N0, M0 T2b, N0, M0
Stage III	T3a, N0, M0 T3b, N0, M0 T4a, N0, M0
Stage IV	T4b, N0, M0 Any T, N1, M0 Any T, N2, M0 Any T, N3, M0 Any T, any N, M1

(Greene et al., 2002)

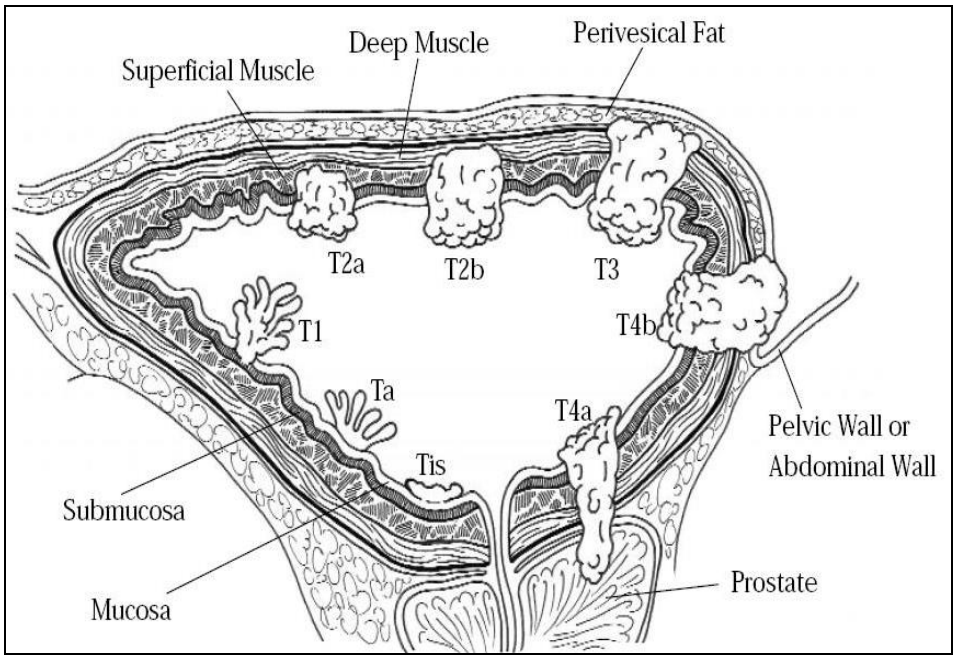

Figure (1): Local tumor (T) staging of bladder cancer ***(Koppie and Bochner, 2006).***

3. Histopathological Classification of Bladder Cancer:

According to ***Messing (2007)***, bladder cancer is classified into urothelial and non-urothelial tumors.

a) Urothelial tumors of the bladder:

i) Carcinoma in situ (CIS):

Carcinoma in situ consists of poorly differentiated TCC confined to the urothelium. It is present in 25% or more of patients with grade 3 superficial tumors, among which 40 to 83% progress to muscle-invasive cancer. It also occurs in up to 75% of grade 3 muscle-invasive cancers.

ii) Transitional cell carcinoma (TCC):

Worldwide, more than 90% of bladder cancers are TCC. In countries such as Egypt, where bilharzial infestation is endemic, it accounts for only up to 25%. TCCs have an increased number of epithelial cell layers with papillary folding of the mucosa, loss of cell polarity, abnormal cell maturation from basal to superficial layers, increased nuclear-cytoplasmic ratio, prominent nucleoli, clumping of chromatin, and increased number of mitoses. All these features are exhibited increasingly from grades 1 through 3. TCCs manifest in a variety of growth patterns, including papillary, sessile, infiltrating, nodular, mixed, and flat intraepithelial growth (CIS).