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Bladder Cancer and Schistosomiasis: Is There a Difference for the Association?

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1. Introduction

Bladder cancer represents a significant worldwide health problem with an estimated 386,300 new cases and 150,200 deaths in 2008 worldwide. The majority of bladder cancer occurs in males and there is a 14-fold variation in incidence internationally. The highest incidence rates are found in the countries of Europe, North America, and Northern Africa (Jemal et al.2011). Smoking and occupational exposures are the major risk factors in Western countries, whereas chronic infection with Schistosoma hematobium (SH) in developing countries, particularly in Africa and the Middle East, accounts for about 50% of the total burden. The majority of bladder cancers associated with schistosomiasis are squamous cell carcinoma **(Figure 1)**.

Although the majority of bladder cancers, present with disease confined to the superficial layer of the bladder wall, approximately 20–40% of the patients will present with or subsequently develop invasive cancer. Bladder cancer is morphologically heterogeneous; more than 90 % of bladder cancer cases are urothelial (UC, transitional cell, TCC) carcinoma, whereas primary squamous cell carcinoma (SCC), adenocarcinoma, small cell carcinoma and other rare tumors are less common (Lopez-Beltran and Cheng, 2006). Urothelial cell carcinoma can present mixed with other malignant components (**figure 2**). These mixed forms of bladder histologies include squamous differentiation (present in 20 - 60% of bladder cancer cases), adenocarcinoma or glandular differentiation (10%), sarcomatoid (7%), micropapillary (3.7%) and lymphoepìtelioma-like carcinoma. About 1 in 25 Western men and 1 in 80 women will be diagnosed with bladder cancer (BC) sometime in their life. In many developing countries, life expectancy is much lower than Westerns, which is one of the reasons why overall BC incidence (not age-specific incidence) is lower in these developing countries (Albertson and Pinkel, 2003). It is associated with substantial morbidity and mortality. History of Tobacco smoking not only increases the incidence of BC, but also it can increase the tumor grade, its size and the number of tumor lesions (Muscheck et al, 2000). Chronic schistosomal cystitis was related for a long period to the development of BC in areas endemic for schistosomiasis like Egypt. In these areas, risk factors are many, including exposure to schistosomiasis, increased smoking rate and exposure to carcinogenic chemicals (Kallioniemi et al, 1992).

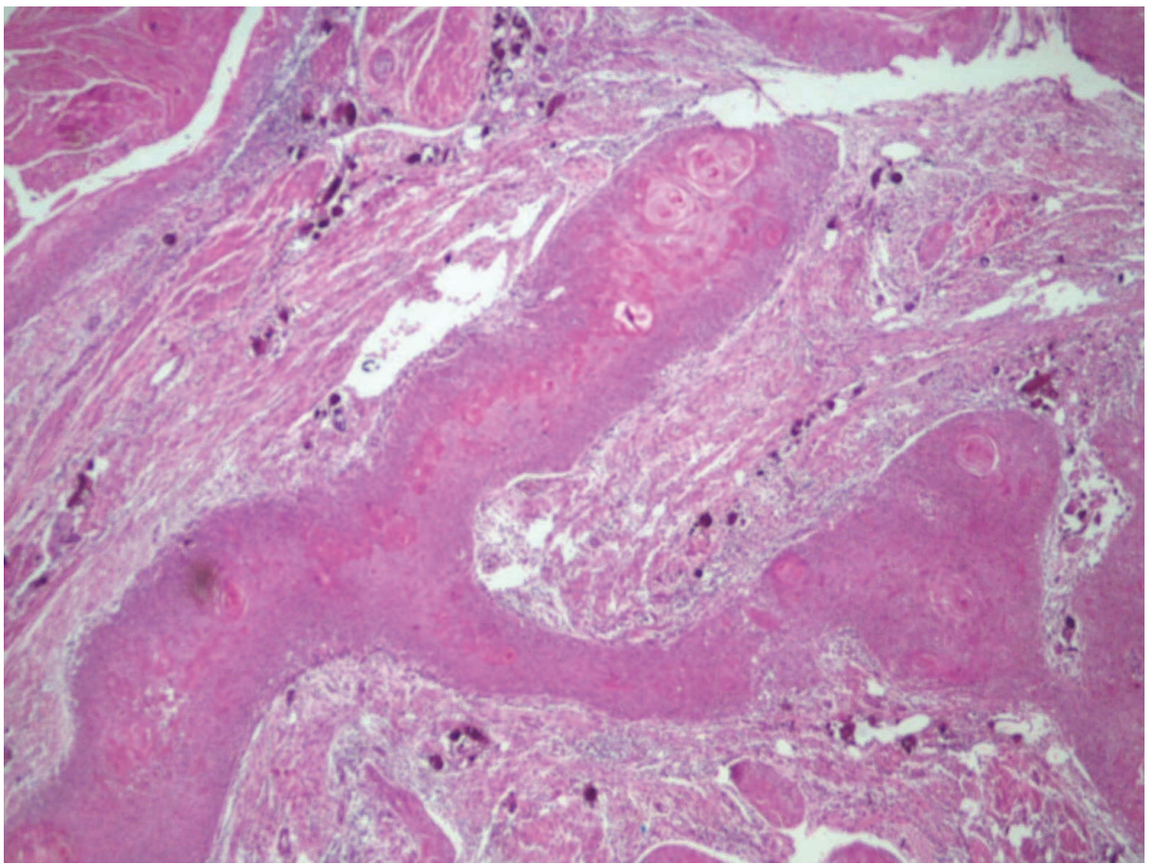

Fig. 1. Squamous cell carcinoma. Groups of malignant squamous cells show central keratin nests formation. Aggregates of calcified bilharzias eggs are seen between groups of malignant cells.

Although smoking is still recognized as a major risk factor of cancers including bladder cancer, the increasing incidence of bladder cancer, despite the reduction in smoking in the United States, suggests that other environmental factors may be playing an increasing role in the development of bladder cancer. Unlike the common belief, risk factors such as positive family history, parent's consanguinity, exposure to pesticides and chronic cystitis seem to play now more important roles than bilharziasis and smoking in the development of this disease in Egypt, yet reports on larger numbers of patients are needed to support this conclusion (Zarzour et al, 2008).

2. Bladder cancer formation

Urothelial tumor is characterized by its multifocality. There have been two theories proposed to explain the frequency of this Urothelial tumor multifocality. One theory, the monoclonal theory, suggests that multiple tumors arise from a single transformed cell that proliferates and spreads throughout the urothelium. The second theory, the field-effect theory, explains tumor multifocality as a development secondary to the field cancerization effect. In the last scenario, carcinogens cause independent transforming genetic alterations at different sites in the urothelial lining leading to multiple genetically defective tumors

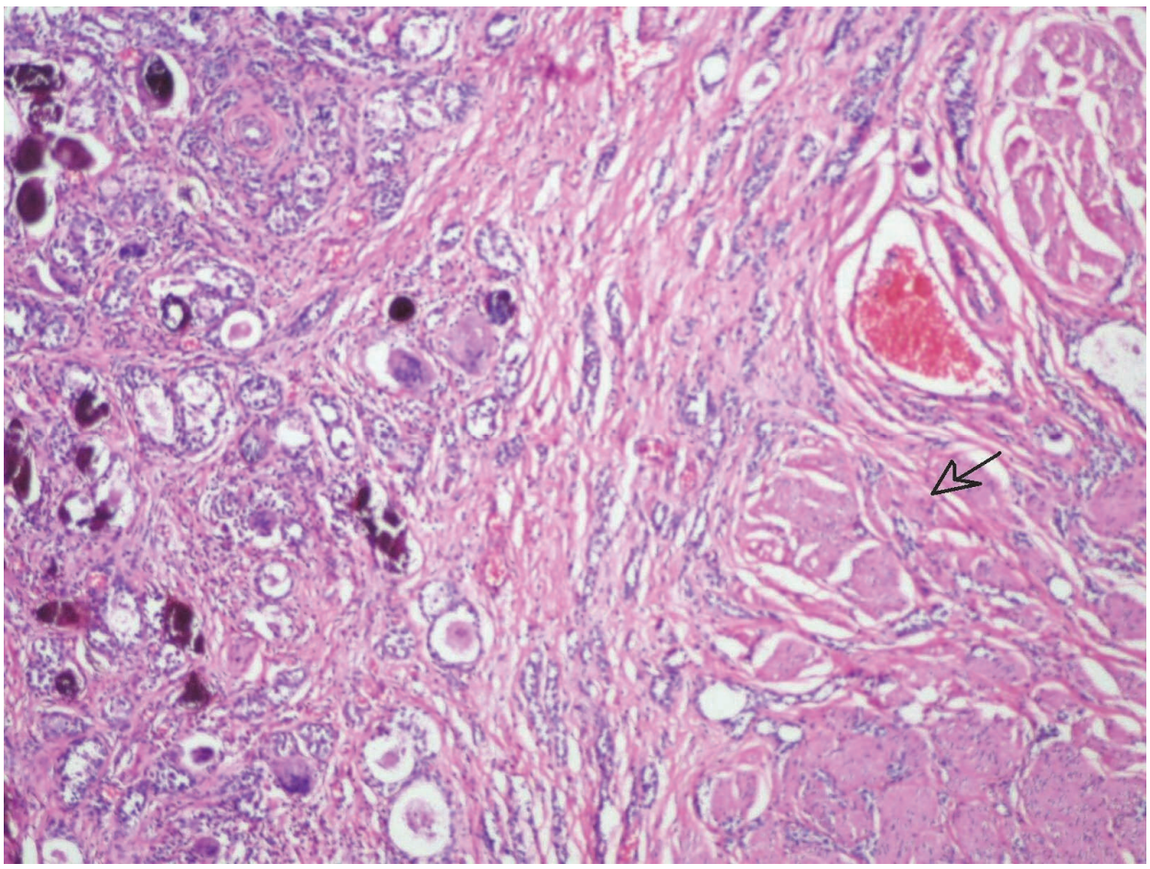

Fig. 2. Invasive urothelial cell carcinoma. Small groups of cells with glandular differentiation on the right are seen infiltrating muscle layer (arrow). Bilharzial granulomas are seen on the left.

(Cheng et al, 2010). That may highlight the existence of different histopathologies in the same specimen. A recent study suggests that both field cancerization and monoclonal tumor spread may coexist in the same patient (Jones et al, 2005). In this study, molecular evidence supported an oligoclonal origin for multifocal Urothelial carcinoma. Field cancerization, which is an important cause of multicenteric squamous cell carcinoma (SCC) of head and neck postulates that multifocal Urothelial carcinoma arises in the same way. The independent transformations are a consequence of external cancer-causing influences. Premalignant changes, such as dysplasia or carcinoma in situ (CIS) are often found in Urothelial mucosa distant from an invasive bladder cancer. Furthermore, various theories have been proposed to combine the two mechanisms. Early or preneoplastic lesions may arise independently with specific clone and pseudomonoclonality (Hafner et al, 2002). The modern carcinogenesis model suggests that malignancy represents clonal expansion of one or a few cancer stem cells that proliferate through asymmetric differentiation and can diversify into heterogeneous cancer cell lineages. Asymmetric differentiation means that following cell division, one daughter cell retains the capacity to divide again and the other daughter cell possesses genetic plasticity, allowing phenotypic variation in the offspring. When tumors arise from Chromosomal Somatic Changes (CSC) of progenitor cells, a specific set of genomics, epigenomic and/ or microenvironment niche alterations is essential for

continued clonal expansion. Therefore, each CSC and its pro/zaxgency possess a unique set of genetic, epigenetic and phenotypic features. Genetic alterations of stromal somatic cells assist CSCs in the niche to promote cancer development and progression (Cheng and Zhang, 2008). Since the sixties of the last century, meaningful chromosomal changes were subsequently reported in human cancer. With the establishment of different new methods, detection of these changes became more apparent and allowed better understanding of the process of evolving of different kinds of cancer. Each new method widened the recognition of karyotypic changes, increasing the resolution of cytogenetic details until the limit of microscopic visualization were almost reached. The evolution of cytogenetics encompasses also molecular approaches such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH) whether metaphasic or array. These techniques have revealed novel and otherwise cryptic rearrangements, as well as providing chromosomal information for cases in which conventional cytogenetic analysis is not possible (Sendberg and Meloni-Ehrg, 2010).

A meta-analysis examining urine markers for surveillance showed that Fluorescence In Situ Hybridization (FISH) test had a median sensitivity of 79% and median specificity of 70% in detecting genetic abnormalities in cells present in urine using FISH (Van Rhijin et al, 2005). The main disadvantages of FISH are the lack of standardization of the criterion for a positive test, the low sensitivity of detecting low-grade tumors, its expense, and the need for specially trained laboratory personnel to perform the test (Degtyar et al, 2004 & Lokeshwar et al, 2005). Combined testing with other assays may improve the effectiveness of this biomarker. Several markers have shown promise as noninvasive biomarkers of bladder cancer, and some may be useful as therapeutic targets. To date, however, none have found a strong niche in clinical care because of the lack of evidence demonstrating that outcomes are altered on a practical basis. In addition, at this time, none of these markers can supplant cystoscopy, and most add little advantage to the combination of cystoscopy and cytology.

3. Schistosomiasis

Schistosomiasis infect 200 million people according to the World Health Organization and is endemic in as much as 76 tropical developing countries. S. hematobium (SH) is associated with bladder cancer. Schistosomes are dioecious parasitic blood flukes, which have a mammalian host and an intermediate invertebrate host: fresh water snails (Kuper et al, 2000). There are four human schistosomes :S. haematobium, S. mansoni, S. japonicum, S. mekonji. The S. haematobium, like other schistosomes is dioecious and the adult female lives in-copulo in the gynecophoral canal of the male; this species of schistosome lives in the venules of the human urinary bladder. Eggs laid in the urinary bladder produce irritation and eventual fibrosis, contributing to the events that lead to human carcinogenicity (Fried et al, 2011).

All schistosoma infections follow direct contact with fresh water that harbors free-swimming larval forms of the parasite known as cercariae. Cercariae penetrate the skin. The cercariae shed their bifurcated tails, and the resulting schistosomula enter capillaries and lymphatic vessels en route to the lungs. After several days, the worms migrate to the portal venous system, where they mature and unite. Pairs of worms then migrate to the vesical plexus and veins draining the ureters. Egg production commences four to six weeks after infection and continues for the life of the worm, usually three to five years. Eggs pass from the lumen of blood vessels into adjacent tissues, and many then pass through the bladder

mucosa and are shed in the urine. The life cycle is completed when the eggs hatch, releasing miracidia that, in turn, infect specific freshwater snails (Bulinus species).After two generations - primary and then daughter sporocysts — within the snail, cercariae are released (Ross et al, 2002).

4. Schistosoma-Associated Bladder Cancer (SA-BC)

Schistosomiasis was first linked to urinary bladder cancer in Egypt in 1911 (Ferguson et al, 1911). The incidence of urinary bladder cancer in the Middle East and Africa is greater in areas with high rather than low SH prevalence; the aforementioned study noted that 60% of the Egyptian population was at risk of infection with SH, with rural school children at particular risk because of their proximity to contaminated water. The overall prevalence of SH infection in Egypt was 37–48% that decreased due to the antibilharzial campaign to 3 % (Ministry of Health and Population, 2004). The urinary bladder cancer accounted for about 31% of the total incidence of cancers in Egypt that subsequently decreased to 12% in recent years. However still, it is the most common type of cancer in males and the second most prevalent, after breast cancer, in females (Gouda et al, 2007). In Egypt, Iraq, Zambia, Zimbabwe, Malawi and Sudan, the incidence of SA-BC peaks at 40–49 years of age; the male to female ratio for bladder cancer is 5:1 in endemic and 3:1 in non-endemic areas. This relates to the fact that it is agricultural workers, mainly men, who have daily exposure to water infected with SH cercariae. (Makhyoun et al, 1971). Mechanicaly, there are several factors that may contribute to the oncological potential of schistosomia infection. Schistosoma ova deposited in the bladder provoke an intense inflammatory reaction, associated with the production of oxygen-derived free radicals, which may induce genetic mutations or promote the production of carcinogenic compounds (such as N-nitrosamines and polycyclic aromatic hydrocarbons) (Marletta 1988 & Rosin et al, 1994) ,leading to malignant transformation. Shokeir (2004) showed that schistosomiasis is often accompanied by chronic bacterial super-infection, which may in itself predispose to squamous cell (SC) neoplasia. Bacteria found to accompany schistosomiasis can promote the formation of N-nitrosation of amines, adding to those from other sources such as the diet. A 54–81% incidence of SCC was found in all cases of bladder cancers in endemic areas, opposed to 3–10% in Western countries. The higher incidence of SCC is probably due to exposure to carcinogens such as N-nitroso compounds that are abundantly present in the urine of patients with SH (Tricker et al,1989). International Agency for Research on Cancer (IARC) found that the intensity of infection was determined by urinary egg counts and confounded by smoking, a recognized cause of bladder cancer in non-endemic countries, and the combination was strongly considered. Positive association between bladder cancer and SH infection was detected, with odd ratios ranging from 2 to 14. The more heavily infected individuals were with this schistosome, the more likely they were to develop bladder cancer, and at a younger age (IARC, 1994).

Most of the pathological findings of schistosomiasis are due to an inflammatory and immunological response to egg deposition. Granulomatous areas form around the eggs and induce an exudative cellular response consisting of lymphocytes, polymorphonuclear leukocytes and eosinophil. The early stage of SH infection is characterized by egg deposition in the lower ureters and urinary bladder. Resultant perioval granulomas, fibrosis and muscular hypertrophy are seen histologically. In the ureter, lesions can cause stenosis, leading to hydronephrosis. In the urinary bladder, masses of large granulomatous

inflammatory polyps containing eggs are found at the bladder apex, dome, trigone and posterior wall. Polyps may ulcerate and slough, producing haematuria. Hyperplasia of the urothelium occurred in 38% of the autopsied SH cases as opposed to 21% in non-infected cases; also, metaplasia in 31.6% versus 11.5% and dysplasia in 27.2% versus 8.5% cases were found. Late-stage infections were characterized by schistosomal bladder ulcers and sandy patches, and irregularly thickened or atrophic mucosa in the posterior bladder or trigone area. Histologically, fibrosis with some round cell infiltration was seen; old granulomas containing calcified or disintegrating eggs were also seen (Smith and Christie, 1986). The inflammatory and fibrotic response to egg deposition could lead to calcification of the urinary bladder, infection and stone disease and these changes are frequently associated with urinary bladder cancer (EL-Bolkainy et al, 1981). These lesions may be at least partially responsible to the reported clinical picture of SA-BC. Furthermore, the following sequence of events in SH-induced carcinogenesis has been suggested: chronic infection leads to schistosoma eggs being trapped in the bladder wall. Proliferation of cells in the bladder mucosa results from constant irritation and inflammation. Clones of neoplastic cells develop, stimulated by N-nitrosamines and other environmental carcinogens such as cigarette smoke and pesticides (Abdel et al, 2000). The importance of urinary retention, whether from fibrosis and obstruction of the urinary bladder neck or from voluntary causes such as pain on urination, in prolonging the exposure of the bladder mucosa to various exogenous and endogenous carcinogens was documented. Schistosome-induced urinary stasis allows increased absorption of carcinogens and therefore plays an integral role in carcinogenesis. Recurrent bacterial urinary tract infections are associated with squamous cell carcinoma of the urinary bladder, even in the absence of SH infection (Genile et al, 1985). Carcinogenesis of SH involving an initiating and promoting effect has been described. First, the damage occurs to the DNA template which, unless repaired, leads to irreversible changes in the complementary strand of DNA produced during the S-phase of the cell cycle. Somatic mutation results when the altered strand is used as a template. The promotion phase followed by stimulation of cell proliferation. Different cancer-associated genes, notably protoncogenes/oncogenes and tumor suppressor genes, were known to be associated with numerous human cancers; recent efforts have been made to study the specific genes involved in the induction of SA-BC. Cell exposed to SH cell total antigen (warm exttract) was found to divide faster than those not exposed to the parasite and died much less. This was probably due to increased level of bcl2, a protein involved in cancer apoptosis that may lead to SH carcinogenic ability in bladder urothelium (Botelho et al, 2009). The urothelium of mice exposed to SH total antigen showed dysplasia, low grade intra-urothelial neoplasm, non-invasive malignant flat lesions in 70 % of the tested mice after 40 weeks of exposure. Carcinoma of the bladder frequently harbors gene mutations that constitutively activate the receptors tyrosine kinase Ras pathway (Wu, 2005). The Ras gene product is a monometric membrane-localized G protein of 21 Kd that functions as a molecular switch linking receptors and non-receptors tyrosine kinase activation to downstream cytoplasmic or nuclear events. Each mammalian cell contains at least three distinct Ras proto-oncogenes encoding closely related but distinct protein, Kras, Hras and Nras. Activating mutation in these Ras protein, result in constitutive signaling. Thereby stimulating cell proliferation and inhibiting apoptosis. Oncogenic mutations in the Ras gene are present in approximately 30% of all human cancer (Adjer, 2001). Botelho et al (2010) used the dysplastic bladders induced by SH in mice and screened them by sequencing for

mutations in Kras codon hotspots gene. They concluded that the parasite abstract has carcinogenic ability possibly through oncogenic mutation of Kras gene.

5. Genetic changes in SA-BC

Among the most common genetic changes in bladder cancer is the loss of heterozygosity (LOH) on chromosomes 9p and 9q, which is found regardless of tumor grade and stage (Jacobs et al,2010 & McConkey et al, 2010). A prospective study stated that there was no evident line of demarcation between schistosomiasis-associated and non schistosomiasis-associated bladder cancer in terms of LOH of microsatellite markers on chromosome 9. This suggests that data obtained from schistosoma-associated bladder cancer can be extrapolated to bladder cancer induced by a schistosomiasis independent mechanism (Abdel Wahab et al, 2005). DNA microsatellites are highly polymorphic repeats found throughout the genome, and microsatellite markers can detect cancer-associated alterations in genetic material, including microsatellite instability and LOH (Nielsen et al, 2006). A more recent analytical tool that has been developed to detect genomic instability in urinary DNA uses small nucleotide polymorphisms (SNPs). SNP chips have a potential advantage over microsatellite analysis in that they can screen more than 300 genetic loci at once compared with 13-20 loci, which leads to a greater sensitivity of the detection of molecular changes (Hoque et al, 2003).

6. Cytogenetics for understanding carcinogenesis

Carcinogenesis is a complex process in which normal cell growth is modified as a result of the interaction of multiple factors, including xenobiotics and endogenous constituents. Carcinogenic process results from the accumulation of both genetic and epigenetic changes that are driven by instability of cellular genome and alterations in inter- or intra-cellular communication, which disrupt the cell proliferation regulation process (Loeb and loeb, 2000). Cytogenetics is concerned with the task of finding recurrent (repeated) or specific abnormalities associated with cancer, and continues to provide crucial diagnostic and prognostic information. In current practice, cytogenetic data often serve as a guide in other studies, ranging from the exploration of cytogenetic findings with various methodologies, singly or in combination, including fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), or microarray-based technologies such as comparative genomic hybridization (CGH), both metaphasic (mCGH) and array (aCGH), to the use of immunohistochemical techniques by the pathologist. Cytogenetic data also provide key background information for the recognition and identification of genes (and their networks) involved in cancer. Progress in understanding the cytogenetic and molecular basis of neoplastic transformation has strengthened the conception of cancer as a genetic disease. Thus, the finding of apparently normal karyotypes in abnormal cells presents an enigma. It can be assumed that cryptic genetic changes are involved in such cases. Newer technologies highlight the more complicated and perplexing aspects of cancer that have eluded more traditional cytogenetic studies. For example, molecular studies have demonstrated fusion genes associated with many tumors like prostate and lung cancers that are not discernible cytogenetically. These findings raise the strong possibility that more epithelial carcinomas, which are usually associated with numerous or complex karyotypic alterations, will be shown to have cryptic primary genetic alterations (Sandberg et al,2010)

7. Requirements and limitations of cancer cytogenetic studies

Cytogenetic techniques require the presence of dividing cells (preferably in the metaphase stage) for the visualization of chromosomes. Thus, fresh specimens are necessary for establishing either short-term or long-term cultures (Sandberg, 1990, Brigge and Sandberg, 2000, Sandberg and Chen, 2001& Gersen and Keagle, 2005). Nevertheless, useful genetic information can be obtained from fixed specimens with appropriate FISH or other molecular techniques (Gersen and Keagle, 2005). Cytogenetic changes represent genetic mechanisms that are thought to be responsible for the biology of the respective clinical conditions, and have become important components of diagnostic and prognostic criteria. There are two different classes of genetic alterations associated with cancer: activation of oncogenes and inactivation of tumor suppressor genes. Rearrangements are a common source of activating mutations. Another scenario is exemplified by chromosome translocations, inversions, or insertions that lead to formation of fusion oncogenes. The oncogene fusion mechanism has received increased attention, because many of these fusions lead to activation of protein tyrosine kinases (PTKs) in various types of cancer. Most of the cytogenetic changes involve activation of receptor proteins, especially PTKs. Receptor PTKs are a highly regulated family of proteins in normal cells, but may undergo activating mutations or structural alterations to become oncoproteins in human malignancies. As already noted, oncogenic activation of PTKs can result from genetic lesions such as point mutations, deletions, or overexpression by gene amplification. Alternatively, chromosomal rearrangements such as translocations, inversions, and insertions that lead to formation of an oncogenic gene fusion can involve receptor PTK or other PTK encoding genes as fusion partners. Another type of gene rearrangement involves tumor suppressor genes, whose products normally serve as brakes on cell growth and runaway cell proliferation. Inactivation of tumor suppressor genes leads to uncontrolled cell proliferation and downregulation of apoptosis (programmed cell death)(Jones and Baylin, 2002 & Feinberg and Tycko, 2004). The activation of oncogenes sometimes results from complex genetic rearrangements. In each of the fusion genes the kinase domain of the neural-associated receptor tyrosine kinase gene is fused to an activating domain of another gene. The same genes may be altered in a number of different tumors, but apparently at varying chronologies in tumor development and associated with different genetic changes. In many tumors, a specific translocation may be the only alteration present. Many cases, however, display additional structural or numeric karyotypic changes that may be responsible for, or at least are associated with, disease progression(Sandberg, 1990). The relevance of additional abnormalities is also reflected by alterations in the expression of a number of genes apart from those involved in the translocations. The exact cause or causes of these additional alterations is unknown, and it remains uncertain whether the primary translocation per se is responsible for the basic genetic process underlying the tumor genesis. These additional changes usually vary from tumor to tumor, even among tumors with the same diagnosis. Tumors with specific translocations may exhibit a variety of anomalies, with or without additional chromosome changes, at the molecular level. Carcinomas being diagnosed relatively late in their development, thus allow for the genesis of chromosomal rearrangements in addition to the primary genetic event. Although some of the chromosomal changes have been related to prognosis and tumor biology yet, few recurrent or repeated chromosomal anomalies have been identified as characterizing these tumors (Teixeira et al, 2006).

MicroRNAs (miRNAs) constitute a rapidly developing field of study at many levels. The miRNAs are short segments of RNA (~22 bases in length) that affect mRNA functions, most often by suppressing translation of the protein product or by promoting degradation of them. An important value of miRNAs is that they can be detected and quantified in a variety of samples, including plasma and formalin-fixed, paraffin-embedded tissues. This makes them a valuable testing tool, particularly in the clinical arena(Wijnhoven et al, 2007, Grady and Tewari, 2010 & Ferracin et al, 2010)

In cancer, the combination of cytogenetic and molecular studies (FISH, SKY, PCR, CGH, and related methodologies) can more clearly define pathogenetic pathways and the biologic functions of molecular markers than either approach alone. Such a dual approach should lead to less empiric and more biologically oriented approaches to tumor classification and, ultimately, to more efficient clinical use of biomarkers (Wang,2002 & Balsara et al, 2002). Findings based on the combination of cytogenetic and molecular approaches have improved the criteria for diagnosis of cancer. The hypothesis that specific clones of spontaneously evolving aneuploidies or karyotypes, rather than specific mutations, generate the individuality of cancers (Fabarius et al, 2008), may apply to at least some of, if not all, the conditions .Cancer development not only depends on genetic alterations but also on epigenetic changes (Jones and Baylin, 2007). These changes modify gene expression through DNA methylation, histone modifications, chromatin remodeling, and/or the expression of noncoding RNA(Esteller, 2007 & Zaratiegui et al, 2007).

Epigenetic gene silencing in cancer was thought to be restricted to focal events that silenced isolated genes (Smith and Costello, 2006). However, recent findings have indicated that epigenetic silencing can extend to a whole chromosomal region and has been reported to involve DNA methylation and/or histone modification in various cancers (bladder, breast, colorectal, and prostate cancer) (Coolen et al, 2010).

The development of cancer is often a multistage process where the disruption of specific subsets of genes can result in cells expressing a malignant phenotype. However, the series of mutations leading to malignancy has only been elucidated for a small number of human cancers (e.g., polyposis of the colon, retinoblastoma). There has been no entirely specific cytogenetic aberration identified for bladder cancer, but various nonrandom deletions, gains of chromosomes, polyploidisation, and formation of isochromosomes have been observed (Gibas and Gibas, 1997).

8. Bladder cancer cytogenetics and epigenetics

Chromosome 1 has been reported as being the most frequently involved chromosome in rearrangements; other chromosomes commonly reported in bladder cancer include chromosomes 3,5,7, and 9 (Heim and Mitelman, 1995). Yunis and Soreng (1984) suggested that there was a relationship between chromosomal fragile sites and oncogenesis. It is believed that fragile sites provide regions of the genome that are more susceptible to damage and that this contributes to the carcinogenic process because of subsequent changes to gene function or dosage. Bladder cancer is a very heterogenous disease cytogenetically, which suggests that the pathogenesis of the disease may not be consistent for every case. A possible scenario of pathogenesis could be the disruption of a nonconsistent set of cell regulatory genes compounded with disruption to genes that have a phenotypic effect on the bladder. Sustained disruptions to fragile regions as a result of prolonged exposure to clastogens in vivo are likely to lead to enduring chromosomal rearrangements and

associated gene alterations. The heterogeneity of cytogenetic findings in bladder cancer hints that there might be different "fingerprints" or accumulations of genetic changes that individually lead to bladder cancer and that fragile sites may be providing a gateway for oncogenesis for some cases. Different combinations of these damaged sites could result in varying cancer phenotypes, depending upon which particular genes were located at the sites susceptible to the mutagens.

Protoncogenes encode proteins that ultimately enhance cell proliferation. Events that convert protoncogenes to oncogenes can lead to uncontrolled cell proliferation and carcinogenesis (Badawi et al, 1995). The RAS oncogene and its potential association with urinary bladder cancer was studied, though still not totally clear. The RAS oncogene encodes a 21-kDa protein that affects signal transmission between the nucleus and tyrosine kinase receptors. H-RAS activation was estimated in bladder cancer to range between 7% and 17%, with its expression being similar with or without concurrent schistosomal infection. The TP53 tumor suppressor gene, located on the short arm of chromosome 17, encodes a protein that regulates DNA damage repair and controls aspects of the cell cycle involving cellular apoptosis and senescence. TP53 mutation results in a reduction of DNA damage surveillance leading to instability of the genome and malignant transformation (Strohmeyer and Slamon, 1994). The overexpression of the BCL-2 gene in SA-BC patients was found to be up-regulated in squamous but not transitional cell cancers of the urinary bladder. Therefore, this BCL-2 overexpression is consistent with the predominance of SCC in SA-BC. Upregulation of this gene overrides programmed cell apoptosis increasing the risk of genomic instability and may interact with various proto-oncogenes facilitating tumorigenesis. Mutations of TP53 were found in 73% of tumors, BCL-2 expression in 32% and abnormalities of both TP53 and BCL-2 in 13%. Loss of the normal reciprocal control mechanism for apoptosis was suggested in the subset of patients with overexpression of both TP53 and BCL-2 (Chaudhary et al, 1997).

Furthermore, cyclooxygenase-2 is overexpressed in SA-BC. The quantitative relationship between cyclooxygenase-2 expression and tumor grade was statistically significant. The cyclooxygenase-2 role in the complex multi-stage process of SA-BC carcinogenesis was proposed: pro-inflammatory cytokines such as interleukin-1, tumor growth factor-β and tumor necrosis factor-alpha, are generated by activated macrophages in the inflammatory lesions. These cytokines and growth factors are potent inducers of cyclooxygenase-2 production. By-products of uncontrolled cyclooxygenase activity together with endogenous genotoxins produce oxidative and nitrosative stress creating lipid peroxidation by-products. Additional mutations are induced: TP53, H-RAS, deletion of p16 and p15, increased epidermal growth factor receptor, c-erb-2 and tumor necrosis factor-alpha. Increased prostaglandin production up-regulates cyclooxygenase-2, decreases killer T-cell activity, increases BCL-2 and glutathione-S-transferase. These changes increase tumorgenicity by decreasing cell apoptosis, creating immunosupression. Prostaglandin products of cyclooxygenase-2 cause tumor progression and eventual metastasis by down-regulating adhesion molecules, increasing the degradation of extracellular matrix and increasing angiogenesis (El-Sheikh et al, 2001).

9. Natural history of SA-BC

The association between SA-BC and SH was established through case-controlled studies and through the close correlation of the incidence of bladder cancer with the prevalence of SH

within different geographic areas. Moreover, the association was based on the frequent association of tumors with the presence of parasitic eggs and egg-induced granulomatous pathology involving bladder tissues *(Figure 2)*. Despite that linkage between SH and bladder cancer, only limited data are available on cytopathologic findings in SA-BC. The cellular mechanisms linking SH infestation with bladder cancer formation are not yet defined. In some cases, severe metaplasia in bladder urothelium may represent a precancerous transformation, whereas in others it may merely serve as a marker of prolonged inflammation, which is associated with high cancer risk (Hodder et al, 2000). Keratinizing or adenomatous metaplasia per se has a strong association with cancer formation in patients with chronic irritation due to bladder stones, chronic infection, or prolonged catheterization.

SA-BC was defined by characteristic pathology (i.e., squamous carcinoma, transitional cell carcinoma, or adenocarcinoma, rather than mainly transitional) and cellular and molecular biology that differ from non-Schistosoma-associated bladder cancer (NSA-BC). Few studies have analysed the cytogenetic and molecular genetic abnormalities in SA-BC and some compared DNA copy number changes in SA-BC and NSA-BC (Kallioniemi et al, 1992,Tsutumi et al, 1998, Muscheck et al,2000, Fadl-Elmula et al, 2002 & Albertson and Pinkel, 2003). Further future studies are needed to characterize the genetic alterations in schistosomal bladder tumors and their role in bladder cancer induction.

These studies used metaphase CGH to obtain overview of chromosomal alterations in SA-BC. The value of pooled DNA in aCGH was shown to be advantageous in detecting recurrent changes associated with specific histopathologic or clinical features (Kendziorski et al, 2005). Two more recent studies used aCGH, rather than metaphase CGH (Armengol et al, 2007 & Vauhkonen et al, 2007). Array CGH provides higher density region-specific coverage and direct mapping of aberrations to the genome sequence, as well as higher throughput (Albertson and Pinkel, 2003). This ensures greater accuracy in comparing two groups of tumors (e.g., SA-BC and NSA-BC). Muscheck et al. (2000) demonstrated deletion similarities in Schistosoma-associated transitional cell carcinoma (SA-TCC) and Schistosoma-associated squamous cell carcinoma (SA-SCC), compared to what has been previously reported by Kallioniemi et al. (1992) on NSA-TCC and Tsutsumi et al. (1998) on NSA-SCC. The previous investigators (Kallioniemi et al,1992,Tsutsumi et al,1998, Muscheck et al, 2000, Fadl-Elmula et al, 2002 & Albertson and Pinkel, 2003) used the technique of CGH on individual tumor tissues, not pooled tissues of similar pathologies. Armengol et al. (2007) used an excellent technique of combining similar pathological types into pools of tissue arising from patients having similar pathological subtypes. These pooled DNAs revealed recurrent primary changes covering secondary changes that vary from case to case. The pooled specimens of SA-BC tumors showed no schistosomiasis specific changes, compared with pools of NSA tumors. The comparison between SA-TCC and NSA-TCC and that between SA-SCC and NSA-SCC gave similar results. DNA copy number profiles of urinary bladder SA adenocarcinoma revealed similarities to that of SA-TCC and SA-SCC reported by Vauhkonen et al. (2007). The results in these two publications showed that the detailed analysis of individual genes revealed a set of genes with the same copy number changes in all bladder carcinomas, including both SA and NSA tumors. Armengol et al. (2007) concluded that there are no major cytogenetic differences among different urinary bladder epithelial tumors, regardless of the suspected carcinogen. All the detected imbalances in SA-BC have been repeatedly reported in NSA-BC that suggested that cytogenetic profiles of

chemical- and Schistosoma-induced carcinoma are largely similar in the reports of Muscheck et al. (2000) and Fadl-Elmula et al. (2002). Patients having SA-BC usually present late with more advanced stage, due to the repeated SH infestations having similar symptoms. The decreased intensity of schistosomal infestation in Egypt led to a changing pattern of the clinicoepidemiologic features of SA-BC. A decreased SCC/TCC ratio (increase in the percentage of TCC and decrease in that of SCC), lowering of the tumor stage and increase in the mean age incidence and percentage of pelvic nodal involvement have been reported. The reported clinicoepidemiologic differences between SA-BC and SNA-BC are now continuously decreasing and the features of SA-BC is slowly approaching that of NSA-BC as reported by Koraitim et al. (1995) and Zaghloul et al. (2008). These changing features were attributed to the decreased intensity of schistosomal infestation in the urinary bladder, as a higher degree of schistosomal infestation and egg deposition was found more frequently with SCC and a lower with TCC (Zaghloul et al, 2008 & Zaghloul, 2010). Furthermore, these changes are repeatedly evident with the predominance of TCC over the SCC type, and a decrease of male predominance. If these changes continue with the same rate, bladder cancer in Egypt is expected to become identical in features to that of Western countries in the near future (Gouda et al, 2007**).**

10. Clinical presentation

Clinical presentations in SA-BC and SNA-BC are similar with few minor differences. Hematuria, dysuria and necroturia are the main symptoms in both situations. However, SA-BC patients usually had experienced these symptoms beforehand as a result of simple schistosomal cystitis. This may be the reason of their relatively late presentation. Table (1) showed the postcystectomy pathological staging in SA-BC and SNA-BC large studies. The early stages (Pa, Pis, P1) were fewer in SA-BC than that in SNA-BC in both the Urothelial and non-urothelial pathology. The pelvic nodal involvement was nearly similar in SA-BC (range: 16.7% - 25.5%) , Urothelial SNA-BC (range: 16.3% - 45%) and non-urothelial SNA-BC (21.8% - 23%). The clincopathologic differences between SA-BC and SNA-BC were previously summarized as late presentation, with younger median age and a higher percentage of squamous cell carcinoma category (Zaghloul, 1994).

11. Treatment of non-muscle invasive (superficial) bladder cancer

Treatment of superficial bladder cancer remains to be transurethral resection and bladder biopsy (TURBT) with and without intravesical BCG or chemotherapy instillation. Although this treatment type is very popular in Urothelial cancer, it is less popular in non-urothelial SNA-BC and SA-BC, probably due to the rarity of the non-invasive stages and the presence of many lesions either precancerous or cancerous in the bladder mucosa.

12. Treatment of muscle-invasive bladder cancer

Radical cystectomy

Muscle-invasive bladder cancer is mostly treated with radical cystectomy in many parts of the world. Radical cystectomy procedure includes removal of the bladder, seminal vesicles and prostate together with perivesical fat and peritoneal coverage, in addition to bilateral

Author	Number of Patients	PTa,is,1 %	PT2 %	PT3 %	PT4 %	Nodal involvement
Pure Urothelial Carcinoma (SNA-BC)						
Bassi et al (1999)	338	32.8	19.8	42.0	19.8	NM
Stein et al, (2001)	1057	39.9	23.5	23.5	13	23.3
Cheng et al, (2003)	303	36.1	28.6	25.5	9.9	16.3
Shariat et al, (2006)	958	22	35	31	12	23
Urothelial & Non-urothelial (SNA-BC)						
Rogers et al, (2006)	955	21	33	32	14	23
Lughezzani et al, (2010)	12003	13.4	38.9	28.1	19.6	21.8
Scosyrev et al,(2009)	1422	14.8	29	29.3	26.8	
Urothelial & Non-urothelial (SA-BC)						
El Said et al, (1997)	420	1	3.8	70.7	24.5	16.7
Zaghloul, (1996)	357	0	33.3	47.9	18.8	24.4
El Makresh et al,(1998)	185	7	25	64	7	16
Zaghloul et al, (2006)	192	3.6	28.1	51.6	16.7	25.5
Ghoneim et, (2008)	2720	10.5	63.9	16.6	9.0	20.4
Zaghloul et al, (2008)	5071	1.9	30.1	54.9	13.1	21.9
Khaled et al, (2005)	180	1.2	5.8	14.1	11.5	16.6
Ali-El-Dein, (2009)	180	10.0	62.8	25.0	2.2	18.3

Table 1. Postcystectomy pathological stages and nodal involvement in pure Urothelial and mixed Urothelial and non-urothelial schistosoma-non associated and schistosoma associated bladder cancer in large series.

endopelvic lymphadenectomy (with varying level of dissection) in male patients. In females, it includes removal of the bladder, its perivesical fat and peritoneal coverage, urethra, uterus, ovary and anterior wall of the vagina (anterior pelvic excentration) (Ghoneim et al, 1997 & Stein et al, 2001). A review of recent literature of treatment results of different types of bladder cancer showed that applying the same treatment yielded nearly the same level of results if comparing the same pathological stage (Zaghloul, 2006 & Zaghloul et al, 2006). Similar 5-year overall survival rates were found in SA-BC, pure Urothelial and combined

Urothelial and non-urothelial SNA-BC types (Table 2). The results were slightly higher in Stein et al. (2001) (NSA-BC) and Zaghloul et al.(2006) (SA-BC) as both studies reported neoadjuvant or adjuvant radiotherapy and /or chemotherapy as a part of treatment in more than one third of their patients. Furthermore, this conclusion applies for comparison of disease-free survival, overall survival, or local control rates for radical cystectomy or even in adjuvant and neoadjuvant radiotherapy types of treatment for SA-BC and NSA-BC (Zaghloul, 2006 & 2010). The treatment end-results of radical cystectomy was not affected by

Author	Patients #	PT1	PT2	PT3	PT4	Nodal involvement
Pure Urothelial Carcinoma (SNA-BC)						
Cheng et al, (2003)	218	---	50	28	17	11
Stein et al, (2001)	1054	74	81/68*	47	44	35
Medersbacher et al, (2003)	507	76	62	40	49	26
Takahashi et al, (2004)	466	81	74	47	38	50
Dhar et al, (2006)	385	---	63	19	NM	9
Ho et al, (2009)	148	77	68	65	11	37
Manoharan et al, (2009)	432	79	60	43	17	22
Urothelial & Non-urothelial (SNA-BC)						
Nishiyama et al, (2004)	1113	82	84/69*	59	43	35
Niu et al, (2008)	356	---	73/44*	22	0	8
Gupta et al, (2008)	502	90	78	70/58*	46	NM
Urothelial & Non-urothelial (SA-BC)						
Ghoneim et al, (1997)	1026	73	66	47/31*	19	23
El Mekresh et al, (1985)	185	83		41		21
Khaled et al, (2005)	180	55		12		6
Zaghloul et al, (2006)	192	100	100/47	40	44	31
Zaghloul et al, (2007)	216	100	51	40	30	31
Ghoneim et al, (2008)	2720	82	75/53	40	30	27

*= a/b, NM = not mentioned

Table 2. The 5-y survival of each pathological stage in Schistosoma-non associated and schistosoma associated bladder cancer patients in large radical cystectomy patients.

the association with schistosomiasis, nor tumor cell type (Urothelial or non-urothelial) in most of the recently published literatures (Ghoneim et al, 1997, Stein et al, 2001, Zaghloul et al, 2006) (Table 2). Favorable end-results were reported for patients with pathologically organ confined disease. These results were constant for both SA-BC (ranged from 47% to 83%), and SNA-BC (ranged from 50% to84%). However, the results were significantly worse when reporting upon locally advanced tumors (PT3N0M0, PT4aN0M0 or Any N). Again, these worse results were experienced by both SA-BC and SNA-BC patients (Ghoneim et al, 1997 & 2008,Stein et al, 2001,Gschwend et al, 2002, Chang et al, 2003, Medersbacher et al, 2003, Nishiyama et al, 2004, Takahashi et al, 2004, Rogers et al, 2006 & Lughezzani et al, 2010). Regardless of the old belief that aberrant differentiation leads to worse results, yet many authors reported similar results of these aberrant variants to UC when comparing stage to stage. Rogers et al (2006) reported a 5-year progression-free survival rate of 60±2% after radical cystectomy for UC and 55±11% for SCC. This difference was statistically insignificant. Patients with UC or SCC had statistically significant higher progression-free survival rates than non-UC non-SCC patients including those having adenocarcinoma. Another study containing considerable number of adenocarcinoma patients was conducted using 17 Surveillance, Epidemiology and End Results (SEER) and it showed a difference of statistical significance in adenocarcinoma patients who underwent RC at a more advanced disease stage than their UC counterparts. Another recent study using a similar SEER database demonstrated that SCC was more aggressive than Urothelial cancer after adjusting for common prognostic factors, such as stage and grade (Scosyrev et al, 2009). Scosyrev et al (2009) concluded that SCC was an independent predictor of mortality among patients with stage III and IV disease, and among patients with stage I and II disease who did not undergo cystectomy as part of their treatment. Therefore, squamous histology was not associated with increased mortality among patients with stage I and II disease when treated with cystectomy. Moreover, Ploeg et al (2010) studied all invasive bladder cancer cases treated in The Netherlands during a 12 year period of (1995-2006). They concluded that the relative survival of muscle-invasive adenocarcinoma patients were equal to that of UC patients. For stage II and III disease, adenocarcinoma patients had even better outcome. Muscle-invasive SCC patients showed worse survival regardless of stage. In SA-BC, Ghoneim et al (2008) demonstrated that SCC (1345 patients) had 10 year overall survival rate (OS) of 53.05% (95% CI: 51-57) compared to 48.49% (44-53 for pure UC (705 patients) and 51.18 % (CI: 45-58) for adenocarcinoma (262 patients). Those patients who had UC with squamous or adenomatous metaplasia (286 patients) showed a lower 10-year OS of 42.78% (CI: 36-49). The lowest 10-year OS was experienced by those patients who had undifferentiated pathology (122 patients) having 10-year OS of 34.23 (CI: 24-45). It is clear from this large-number single institution study that the OS of SCC, UC and adenocarcinoma were similar and having the same profile. They demonstrated that although the univariate analysis was significant (Undifferentiated carcinoma had much lower OS), the multivariate analysis proved that tumor cell type is not an independent working factor determining the OS. The only significant prognostic factors were stage, grade and pelvic nodal involvement. Many authors cautiously concluded that RC treatment end-results were not affected by tumor histology or etiology but affected by other prognostic factors like stage, grade, nodal involvement, lymphovascular invasion, angiogenesis, P53, P21, Retinoblastoma genes (Rb) and other biological factors. These prognostic and predictor factors were shown in many SA-BC and SNA-BC studies to have varying weight effect (Ghoneim et al, 2008,Scosyrev et al, 2009,Ploeg et al, 2010 & Zaghloul, 2010).

13. Preoperative and postoperative radiotherapy

The rationale of preoperative radiotherapy is to prevent intraoperative seeding of tumor cells in the operative field and to sterilize microscopic extensions in the perivesical tissues. In the English literature, there are 6 randomized trials addressing the issue of adding preoperative radiotherapy to RC. Two of these 6 studies were on SABC (Awwad et al, 1979 & Ghoneim et al, 1985). Only one (Awwad et al, 1979) showed the benefit of adding preoperative radiotherapy. Most of the other 5 studies showed this effect on high stage and high grade tumors. On the other hand, there were no differences in statistical values in earlier cases. Meta-analysis of these randomized studies showed a corrected odd ratio of 0.94 (95% CI: 0.57-1.55), indicating no benefit for adding preoperative radiotherapy in BC (Huncharek et al,1998).

Postoperative radiotherapy (PORT) has the advantage of dealing with microscopic cells that are easier to sterilize. It allows better identification of the group of patients that may benefit from this adjuvant therapy. One large prospective randomized trial proved the benefit of PORT in locally advanced SA-BC. The 5-year disease-free survival (DFS) rate was 49 and 44 % for hyperfractionated (HF) and conventional fractionation (CF) PORT, respectively compared with 25% for cystectomy alone patients (Zaghloul et al,1992). This effect was constant across all tumor cell type, all muscle-invasive stages and grades in SA-BC. These results were replicated in a non-randomized prospective controlled Radiation Therapy Oncology Group (RTOG) trial on SNA-BC (Reisinger et al,1992). The results ot the 2 studies were nearly identical when compared stage by stage (Zaghloul, 1994). The only difference was the high GIT late complication rate reported by Reisinger et al study. They reported 37% (15 out of 40 patients) developed intestinal obstruction after PORT. Nine out of these 15 patients required surgery and 3 died. On the contrary, Zaghloul et al (1992) reported 5% and 18% all grades of late GIT complications for the HF and CF respectively. Only 4% and 5% out of the HF and CF group respectively necessitated surgical interference. Similar low levels of late GIT complications were experienced by other retrospective studies reported on SA-BC and SNA-BC (Cozzarini et al,1999, Zaghloul et al,2002, Zaghloul et al, 2006).

Abdel Moneim et al (2011) compared, in a prospective randomized trial, preoperative and postoperative radiotherapy in SA-BC. They administered the same dose of 50 Gy in 5 weeks to both groups. The study reported both similar treatment end-results and late complication rates for the two randomized pre- and postoperative groups.

14. Neoadjuvant and adjuvant chemotherapy

Neoadjuvant and adjuvant chemotherapy have been utilized in bladder cancer, in an attempt to improve the outcome for patients with high risk muscle-invasive disease. At least 50% of these patients developed distant metastasis after radical cystectomy. Several meta-analysis of prospective, randomized trials indicated that patients undergoing neoadjuvant chemotherapy with methotrexate, vinblastine, doxorubicin and cisplatin (MVAC) prior to cystectomy have an approximate 5.0 – 6.5 % survival advantage over those who underwent surgery alone (Winquist et al, 2004 & Vaughn et al, 2005). However, some investigators still argue that this neoadjuvant advantage is small and chemotherapy might be better targeted to those at highest risk of relapse after surgery. Furthermore, many elderly patients or who have comorbidities will not tolerate MVAC chemotherapy. Therefore, many investigators tried adjuvant chemotherapy in a supposed more favorable situation. In reality, adjuvant

chemotherapy yielded a modest, statistically significant improvement in survival over cystectomy alone (Vale, 2006 & Ruggeri et al, 2006).
The Egyptian bladder cancer cooperative group compared Neoadjuvant chemotherapy using gemcitabine-cisplatin regimen to cystectomy alone in 109 SA-BC patients, in a prospective controlled randomized study. The one-year survival rate was 54% for the cystectomy alone patients compared to 69% for the neoadjuvant chemotherapy patients (Khaled et al, 2003).

15. Bladder preservation trimodality treatment

Since the late 1980s, many centers investigated the bladder preservation strategy as an alternative to radical cystectomy. The rationale of this strategy depends on 3 goals: first, eradication of the local disease, second, elimination of potential micrometastasis and third, maintenance of the best possible quality of life (QoL) through organ preservation (Rodel, 2004). Several treatment protocols were carried out by different investigators. However, they all characterized 3 main and essential procedures with varying timing and varying minute details. The first main procedure is maximal TURBT. This is to be followed by neoadjuvant chemotherapy or radiochemotherapy (second procedure) and then after cystoscopic assessment, followed by either radical radiotherapy or consolidation radiochemotherapy for the complete responders (third procedure). There was another group treated with radiochemotherapy after TURBT. Cystoscopic assessment will segregate the complete responder (CR) for bladder-conserving management and those showing less than CR to undergo salvage cystectomy (Zaghloul and Mousa,2010). The 5-year OS rates ranged between 39% and 58% and the 5-year survival with native bladder preservation ranged from 36% to 43% (Tester et al, 1993,Kachnic et al, 1997, Shipley et al, 1998, Sauer et al, 1998 & Arias et al, 2000). Saba et al (2010) reported similar results for UC (SA-BC and SNA-BC) in Egypt using a trimodality treatment. Complete remission was achieved in 79% of cases after initial radiochemotherapy using gemcitabine- cisplatin regimen. The 5-year OS rate for patients with initial CR was 68% which is comparable to the results in SNA-BC in the western countries treated with trimodality therapy. Moreover, Sabba et al (2010) found that the association with schistosomiasis had no significant impact on the results of therapy for their patients.

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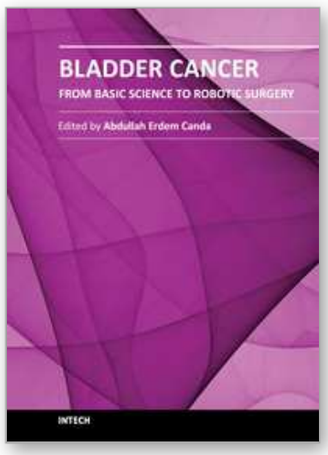

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This book is an invaluable source of knowledge on bladder cancer biology, epidemiology, biomarkers, prognostic factors, and clinical presentation and diagnosis. It is also rich with plenty of up-to-date information, in a well-organized and easy to use format, focusing on the treatment of bladder cancer including surgery, chemotherapy, radiation therapy, immunotherapy, and vaccine therapy. These chapters, written by the experts in their fields, include many interesting, demonstrative and colorful pictures, figures, illustrations and tables. Due to its practicality, this book is recommended reading to anyone interested in bladder cancer.

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