TUMOR SUPRESSOR GENE (P53) IN URINARY BLADDER CARCINOMA, A PATHOLOGICAL AND IMMUNOHISTOCHMICAL STUDY

Submitted for partial fulfillment of master degree pathology

By

Saad Moftah Albakoosh

M.B.B.Ch

Under supervision of

Professor Omran Alfituri

Professor of Pathology

Faculty of medicine
Benghazi University

Dr. Adam Elzagheid

M.B.B.Ch, PhD

Assistant professor

Faculty of medicine
Benghazi University

Benghazi_libya

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بكالوريوس طب وجراحة

تحت اشراف

أ.د عمران المهدى الفيتورى

قسم علم الامراض

جامعة بنغازى-كلية الطب

د. أدم ابراهيم الزغيد

قسم علم الامراض

جامعة بنغازى-كلية الطب

بنغازى-ليبيا

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List of abbrevations :

Ac	Adnocarcinoma.
AJCC	American Joint Committee on cancer
ASR	Age standardized rate.
ANOVA	Analysis of variance .
BCR	Benghazi Cancer Registry.
CIS	Carcinoma in citu.
CT	Computed tomography.
DNA	Deoxyribonucleic acid.
FGFR	Fibroblast growth factor receptor.
GSTM1	Glutathione S-transferase M1
H&E	Heamatoxyline &eosine.
Ig	Immunoglubine.
IHC	Immunohistochemistry.
ISUP	International Society of Urological Pathology.
IVU	Intravenous urography.
LMP	Low malignant potential.
LSAB	Labelled streptavidin biotin.
MRI	Magnetic resonance imaging.
PBS	phosphate-buffered saline.
PUNLMP	Papillary urothelial neoplasm of low malignant potential.
Scc	Squamous cell carcinoma.
Tcc	Transitional cell carcinoma.
TURBT	Transurthral resection of bladder tumor.

Ub	Urinary bladder.
UICC	International Union against Cancer.
WHO	World Health Organization.

Aim of the study:

1. Re-evaluation of the histopathologic types and grading of a sample of urinary bladder carcinoma in Libyan patients by the H&E stain.

2. Detection of the extension of invasion and spread of these urinary bladder carcinomas by clinicopathological correlation using the TNM classification.

3. Immunohistochemical staining of one of the prognostic urinary bladder tumor marker (Tp53) & correlation of the results with the available clinicopathologic parameters of urinary bladder carcinoma.

Chapter 1

Introduction

INTRODUCTION

The urinary bladder is the most common site of urinary tract tumors, Bladder cancer may be encountered at any age, but most patients (80%) are 50 to 80 years old. Men are affected three times as often as women. Most tumors are microscopically classified as urothelial (transitional cell) neoplasms, squamous cell carcinoma, adenocarcinomas, neuroendocrine carcinomas are minority. Most tumors are malignant, but their aggressiveness and prognosis vary, depending on the clinical stage and microscopic grade and type of each tumor (Robbin&Cortan, 2007).

Tumors are often multifocal and can occur in any part of the urinary tract lined by transitional epithelium, from the renal pelvis to the posterior urethra. These tumors are believed to arise as a consequence of irreversible damages of the DNA (initiation), then continued division and proliferation (promotion). Progression toward neoplasia may require cumulative effects of one or more initiating/ promoting agents. Molecular genetics and immunopathologic analysis of bladder cancer have identified a number of abnormalities in some of the genes and proteins that have been implicated in the development and progression of such tumors, mainly in the Tp53 pathway (Ryan KM A et al, 2001).

In general, individual prognosis of infiltrating bladder tumors can be poorly predicted based on clinical factors alone. Tumor multifocality, tumour size of >3 cm, and concurrent carcinoma in situ have been identified as risk factors for recurrence and progression (Rodriguez- Alonso A et al, 2002). Tumour extension beyond the bladder on bimanual examination, infiltration of the ureteral orifice, lymph node metastases and presence of systemic dissemination are associated with a poor prognosis (Haleblian GE et al, 1998).

Morphologic prognostic factors include grade, stage, as well as other specific morphologic features. Histologic grade probably has prognostic importance for pT1 tumors. As most pT2 and higher stage tumors are high grade, its value as an independent prognostic marker

remains questionable. Depth of invasion, which forms the basis of pT categorization is the most important prognostic factor. In efforts to stratify category pT1 tumors further, sub-staging systems have been proposed on the basis of the level of invasion into the lamina propria.Tumors that infiltrate beyond the muscularis mucosae have a higher progression rate (Hasui Y et al, 1994).

Despite marked differences in the prognosis of pT1 and pT2-4 cancers, these tumors are highly similar on the genetic level, It could therefore be expected, that similar genetic alterations might be prognostically relevant in all stages. A multitude of molecular features has been analyzed for a possible prognostic role in invasively growing bladder cancer. Despite all this extensive research, there is currently no molecular parameter that is sufficiently validated and has sufficient predictive power to have accepted clinical value in these tumors (Kausch I & Bohle A, 2002).

The tumor suppressor gene (Tumor protein P53) Tp53 is located on chromosome 17p13 and encodes a nuclear phosphoprotein involved in the cell cycle that allows cellular DNA repair and/or apoptosis, which occurs by controlling cellular progression from the G1 to the S phase (Greenblatt Ms et al, 1994). Mutant Tp53 has a prolonged half-life and can be demonstrated by immunohistochemical techniques . Furthermore, over-expression of Tp53 has been correlated with the grade of bladder cancer (Sarkis AS et al, 1995).

TP53 Alterations of the TP53 tumour suppressor gene have been by far the most intensively studied potential prognostic marker . Early studies suggested a strong prognostic importance of immunohistochemically detectable nuclear TP53 protein accumulation in both pT1 and pT2-4 cancers (Sarkis AS et al ,1993, Schmitz-Drager BJ et al, 2000).

A series of 50 libyan patients with urothialial carcinoma were retrospectively studied.all carcinomas were selected from the archive of the department of pathology, derived from the period from 2007 to 2011, based on the availability of representative paraffin blocks.an experienced pathologist confirmed all histological diagnoses. All tumors were classified using

the histopatholgical criteria of the world health organization (WHO) classification, and staging was made according to the American joint committee on cancer system.

The aim of this study is to quantify the tumor gene (TP53) status in urinary bladder cancer & determine whether they correlate with tumor pathologic stage & grade. The material were include about 50 urinary bladder cancer cases which were graded & staged. The specimens were be analyzed for Tp53 immunohistochemical staining by anti Tp53 antibodies. The correlation between histopathological grade & tumor stage will be evaluated.

Chapter 2

Review of Literature

Urinary Bladder

The urinary bladder, an epithelial-lined hollow viscus with strong muscular wall, is characterized by its distensibility, it can accommodate up to 400-500 ml of urine with out achange in intraluminal pressure. The urinary bladder is a temporary reservoir of urine that varies in size , shape , position , and relations according to its content and state of neighboring viscera

Embryology of urinary bladder

The cloaca is divided by the urorectal septum into a dorsal rectum and a ventral urogenital sinus. It is this urogenital sinus that will give rise to the majority of the urinary bladder; it is aided by the caudal migration of the cloacal membrane, which will close the infraumbilical portion of the abdominal wall. The caudal portions of the mesonephric ducts become dilated and eventually fuse with the urogenital sinus in the midline dorsally, contributing to the formation of the bladder trigone. While these ducts contribute initially to the formation of the mucosa of the trigone, this is subsequently entirely replaced by endodermal epithelium of the urogenital sinus. The gradual absorption of the mesonephric ducts bring about the separate opening of the ureters into the urinary bladder in the area of the trigone. During embryologic development, the allantois regresses to completely form a thick, epithelial-lined tube, the urachus, which extends from the umbilicus to the apex (dome) of the bladder. (Figure2-1) (Moore K, 1982; Kissane JM, 1989).

Before or shortly after birth, the urachus involutes further becoming simply a fibrous cord. Pathologists commonly refer to this fibrous cord, which extends from the dome of the bladder to the umbilicus, as the urachal remnant, but it should be called the median umbilical ligament since aeoeurachal remnantae refers to remnants of the epithelial lining of the urachus that occasionally persist within the median umbilical ligament (Moore K, 1982).

The epithelial lining of the urachus is urothelium, similar to that of the urinary bladder and ureter, but it frequently undergoes metaplastic change that is mostly of a glandular nature urogenital sinus in continuity with the allantois. The lamina propria, the muscularis propria, and the adventitia develop from the adjacent splanchnic mesenchyme. These facts are important in understanding the histogenesis and nomenclature of lesions arising from the epithelial surface, as well as the bladder wall. For example, glandular features within benign (cystitis glandularis, nephrogenic. The epithelium of the urinary bladder is endodermally derived from the cranial portion of the adenoma) and malignant (adenocarcinoma) urothelium is not due to mesodermal within the trigone but come about through a process of metaplasia and are a reflection of histologic plasticity (multipotentiality) of the urothelium. Since the mesonephric ducts involute totally during embryologic development, it is wrong to refer to tumors with mixed epithelial and sarcomatoid features arising in the bladder epithelium as mesodermal mixed tumors. They are, in fact, endodermal mixed tumors and are usually called sarcomatoid carcinomas (Eble JN, 2004 etal).

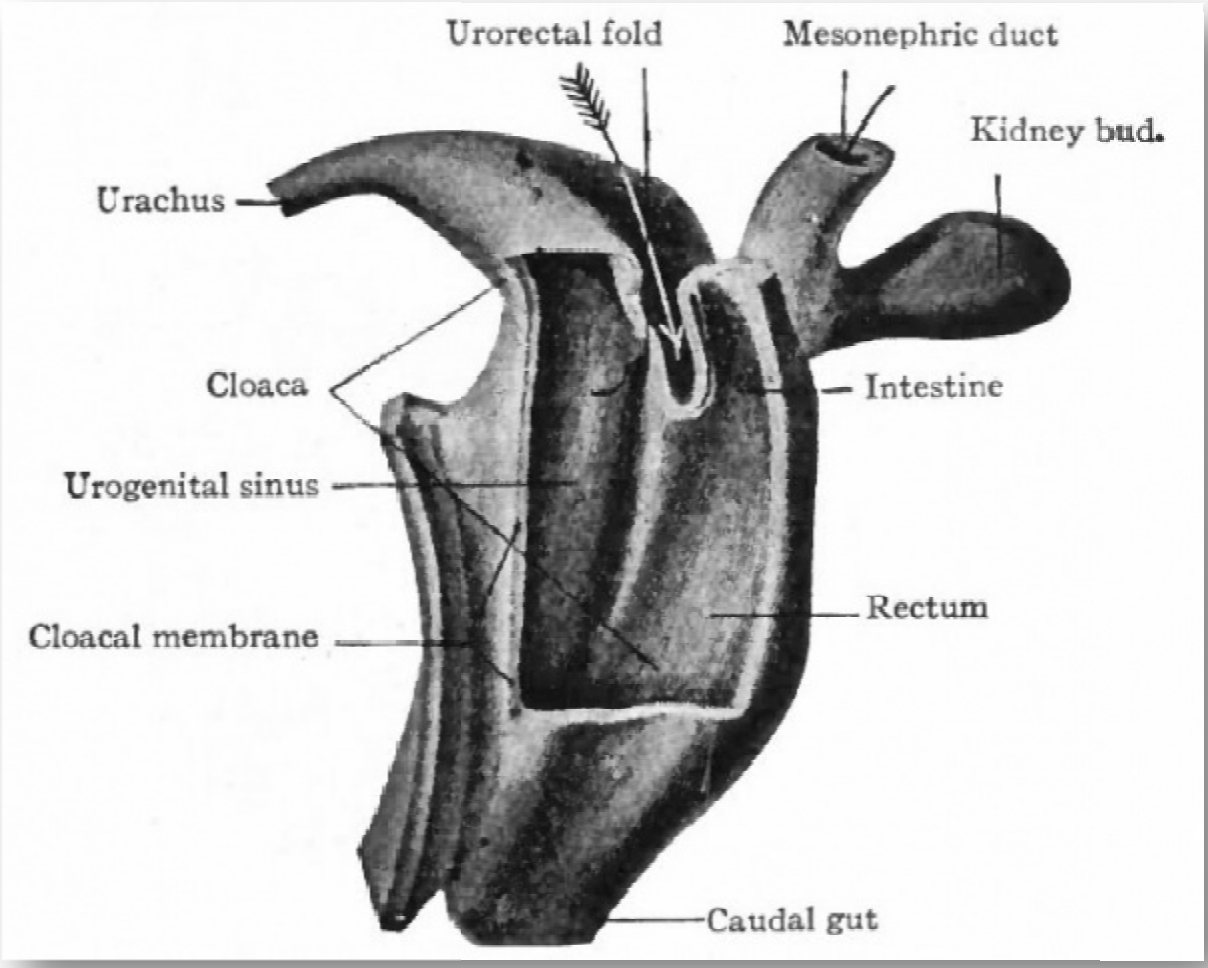

Figure (2-1): Embryology of urinary bladder

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Anatomic Considerations:

Bladder:

In the adult, the empty urinary bladder lies within the anteroinferior portion of the pelvis minor, inferior to the peritoneum. In infants and children, it is located in part within the abdomen, even when empty . It begins to enter the pelvis major at about 6 years of age and will not be found entirely within the pelvis minor until after puberty. Nevertheless, in adults, as the bladder fills it will distend and ascend into the abdomen, at which time it may reach the level of the umbilicus (Moore KL, 1985).

The bladder lies relatively free within the fibrofatty tissues of the pelvis except in the area of the bladder neck, where it is firmly secured by the pubovesical ligaments in the female and the puboprostatic ligaments in the male. The relative freedom of the rest of the bladder permits expansion superiorly as the viscus fills with urine (Tanagho E, 1992).

The empty bladder in an adult has the shape of a four-sided inverted pyramid and is enveloped by the vesical fascia . The superior surface faces superiorly and is covered by the pelvic parietal peritoneum (Figures2-2, 2-3) . The posterior surface, also known as the base of the bladder, faces posteriorly and inferiorly. It is separated from the rectum by the uterine cervix and the proximal portions of the vagina in females and by the seminal vesicles and the ampulla of the vasa deferentia in males. These posterior anatomic relationships are very important clinically. Since the majority of vesicle neoplasms arise in the posterior wall adjacent to the ureteral orifices, invasive tumor may extend into adjacent soft tissue and organs (Moore KL, 1985).

The intimate relationship to the previously mentioned organs explains why hysterectomy and partial vaginectomy are commonly performed at the time of radial cystectomy in women. Similarly, we know that perivesical and seminal vesicle involvement is a bad prognostic sign in bladder carcinoma in males , a reflection of high pathologic stage (Utz DC et al, 1980; Mahadevia PS et al, 1986; Ro JY et al ,1987).

It is important to note that seminal vesicles may contain carcinoma without invasion, and this occurs in cases of in situ urothelial carcinoma involving prostatic and ejaculatory ducts and extending into the seminal vesicle epithelium. The latter is a rare occurrence, but these patients do not appear to have a similarly bad prognosis unless prostatic stromal invasion is present. The two inferolateral surfaces of the bladder face laterally, inferiorly, and anteriorly and are in contact with the fascia of the levator ani muscles. The most anterosuperior point of the bladder is known as the apex, and it is located at the point of contact of the superior surfaces and the two inferolateral surfaces. The apex (dome) marks the point of insertion of the median umbilical ligament and consequently is the area where urachal carcinomas are located (Meyers FH et al, 1968; Tanagho EA et al, 1968).

The trigone is a complex anatomic structure located at the base of the bladder and extending to the posterior bladder neck. In the proximal and lateral aspects of the trigone, the ureters enter into the bladder (ureteral orifices) obliquely. The muscle underlying the mucosa in this region is a combination of smooth muscle of the longitudinal layer of the intramural ureter and detrusor muscle . The intramural ureter is surrounded by a fibromuscular sheath (Waldeyer's sheath) that is fused into the ureteral muscle. This fibromuscular tissue fans out in the area of the trigone and mixes with the detrusor muscle, thus fixing the intramural ureter to the bladder. As the bladder distends, the surrounding musculature exerts pressure on the obliquely oriented intramural ureter, producing closure of the ureteral lumen and thus avoiding reflux of urine (Elbadawi A, 1972; Politano VA, 1972; Shehata R, 1977).

The most distal portion of the bladder is called the bladder neck, and it is marked by the area where the posterior and the inferolateral walls converge and open into the urethra. In the male, the bladder neck merges with the prostate gland, and one may occasionally observe several prostatic ducts present in this area. It is important to recognize the existence of these ducts since their involvement by carcinoma should not be mistaken with invasive carcinoma. The bladder neck is formed with contributions from the trigonal musculature (inner longitudinal ureteral muscle and Waldeyer's sheath), the detrusor musculature, and the urethral musculature. The internal sphincter is located in this general area, with major contributions from the middle circular layer of the detrusor muscle (Tanagho EA, Smith DR ,1966).

The bladder bed (structures on which the bladder neck rests) is formed posteriorly by the rectum in males and vagina in females . Anteriorly and laterally it is formed by the internal obturator and levator ani muscles, as well as the pubic bones. These structures may be involved in advanced tumors occupying the anterior, lateral, or bladder neck regions and render the patient inoperable (Moore KL & Dalley AF, 2005).

Arterial supply of the bladder:

The main arterial blood supply of the bladder comes from the inferior vesical arteries that branch from the internal iliacs. The umbilical arteries and its branches (the superior vesical arteries) also supply the bladder, as do the obturator and inferior gluteal arteries and, in females, the uterine and vaginal arteries (Weiss L, 1988).

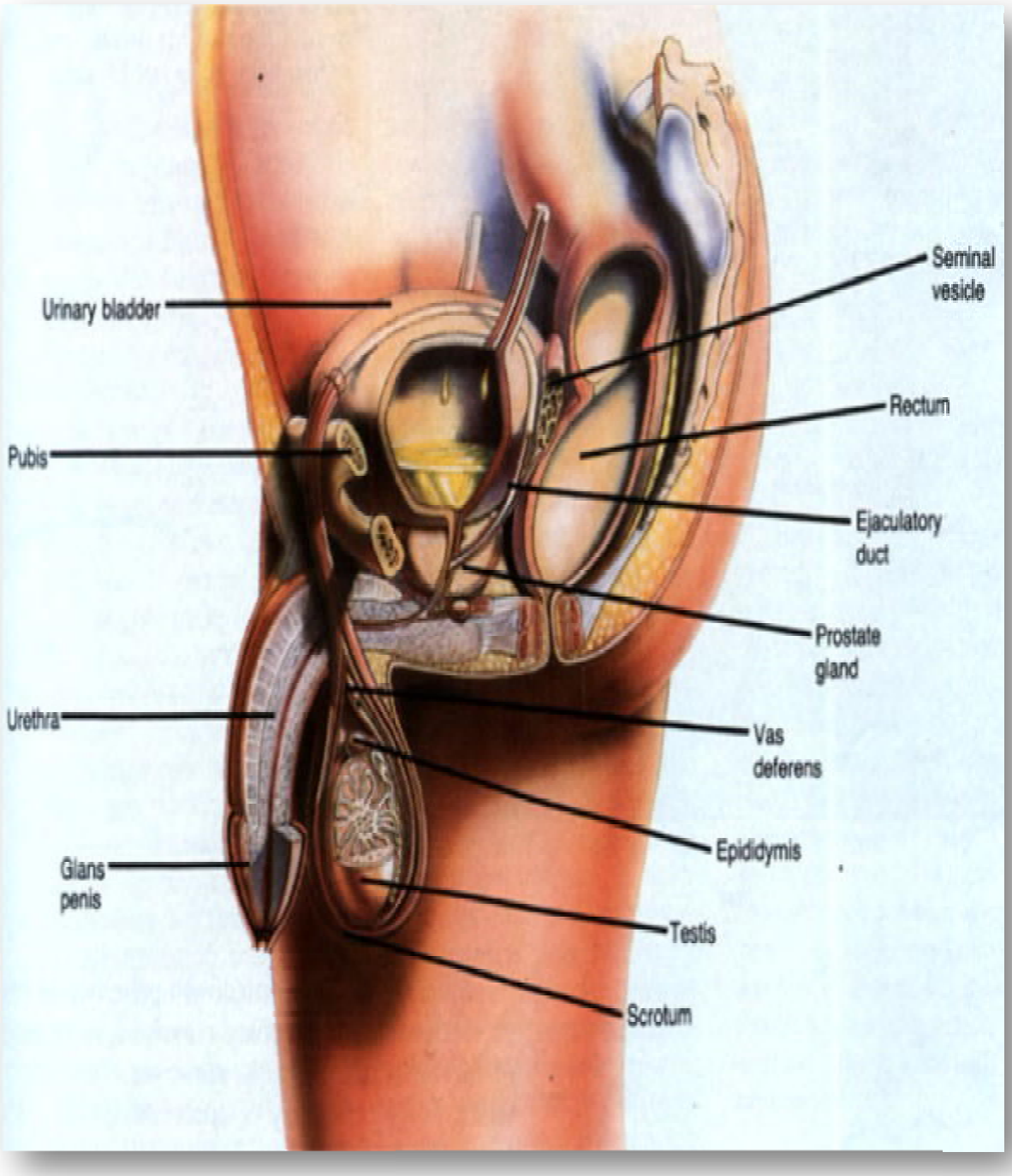
Venous and lymphatic drainage of the bladder:

The veins of the urinary bladder drain into the internal iliac veins and form the vesical venous plexus. In the male, this plexus envelops the bladder base, prostate, and seminal vesicles and connects with the prostatic venous plexus. In females, it covers the bladder neck and urethra and communicates with the vaginal plexus. Lymphatic drainage is through the external and internal lymph nodes although drainage of portions of the bladder neck region may be through the sacral or common iliac nodes (Weiss L, 1988).

Innervations of the bladder:

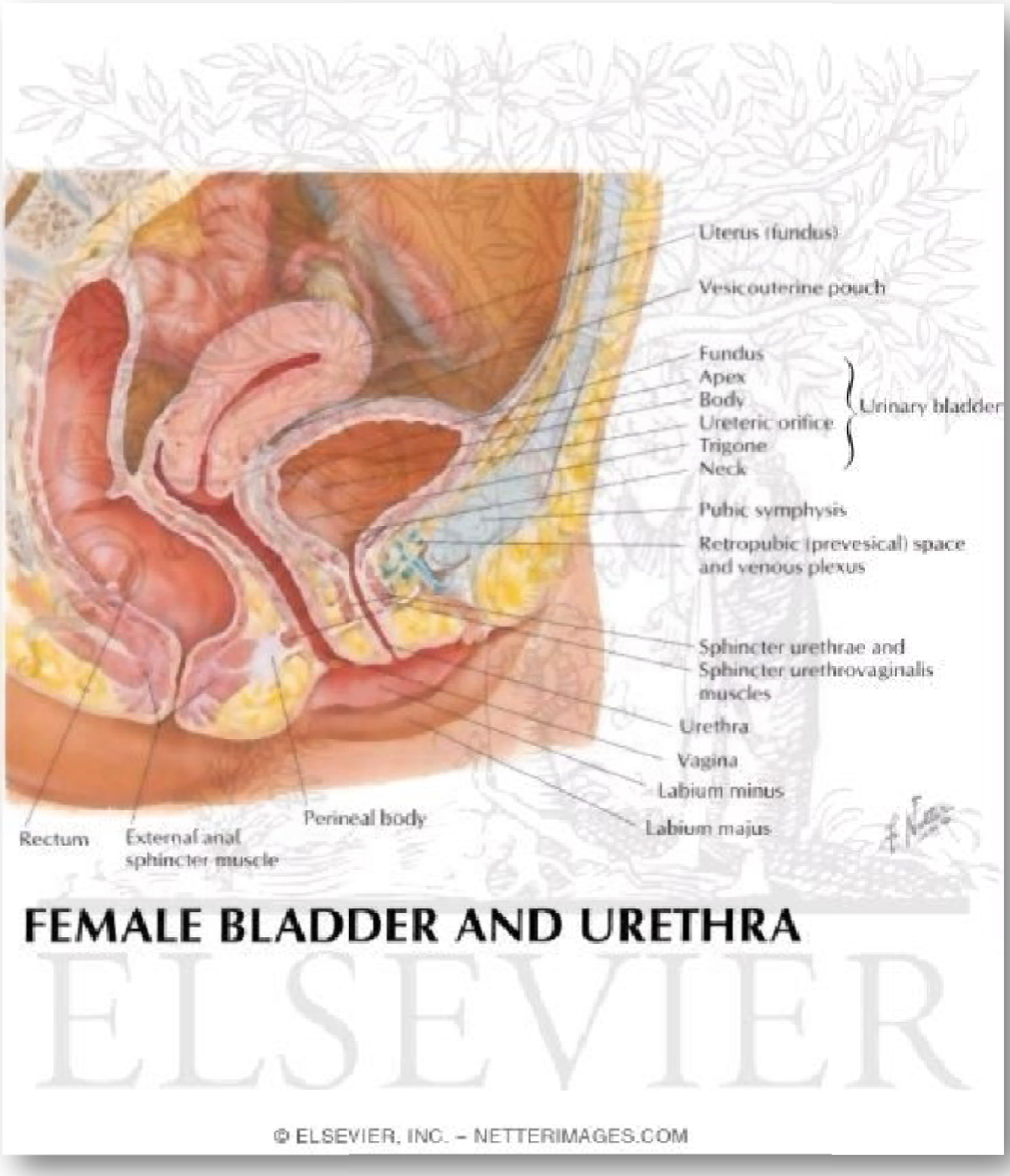
The urinary bladder is supplied by both sympathetic and parasympathetic nerves, which form the vesical nerve plexus. The former are derived from T_{11} through L_2 nerves and play no role in micturition. On the other hand, the parasympathetic nerves come from S_2 through S_4 and travel to the bladder via the pelvic nerve and inferior hypo gastric plexus. These nerves are important to micturition since they contract the fibers of the muscularis propria, which in turn produces traction upon the bladder neck and opens the internal sphincter of the bladder. In fact, it is

believed that micturition is initiated by voluntary relaxation of the perineal muscles and the striated muscle of the external sphincter located along the urethra. This action decreases urethral resistance and triggers contraction of the smooth muscle of the trigone and remaining bladder, closing the ureteral orifices and increasing the hydrostatic pressure within the viscus. These facts account for the difficulty in starting micturition while whistling. The bladder also contains sensory nerves that travel along the pelvic and hypogastric nerves and account for the sensation of pain as the bladder becomes too distended (Fletcher TF & Bradley WE, 1978).



Figure(2-2): anatomy of male urinary bladder

(Clinical Anatomy, 2003)



Figure(2-3): Anatomy of female urinary bladder

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Histology of urinary bladder:

Inner most layer being an epithelial lining and extending outward alamina propria, smooth muscle (muscularis propria) ,and adventitia .The superior surface of the bladder comes in contact with parietal peritoneum hence has aserosa (figure 2-4) . The anatomic and histologic landmarks are used clinically and pathologically to stage patients with urothelial cancer in order to choose therapy and estimate survival (mills& stacyE 2007).

Urothelium:

The urinary bladder is lined by so- called transitional epithelium. This name was coined because its histologic appearance was transitional between non-keratinizing squamous and pseudostratified columnar (Figure2-5). Many histologists and pathologists have suggested urothelium as a more appropriate term. The thickness of the urothelium will vary according to the degree of distension and anatomical location. It may be only two or three cell layers thick along the minor calyces of kidney. In the contracted bladder, it is usually six to seven cells thick and in the ureter three to five cells thick. One can identify three regions: the superficial cells that are in contact with the urinary space, the intermediate cells, and the basal cells that lie on a basement membrane (Koss LG, 1975; Fawcett DW, 1986).

In the distended bladder, the urothelium may be only two to three cells thick and flattened with their long axis horizontal to the basement membrane. In practice, the thickness of the urothelium is dependent not only in the degree of distension but also on the plane on which the tissue is cut. If the cut is tangential to the basement membrane, it is possible to generate an artificially thick mucosa. For these and other reasons, we feel that urothelial thickness is of marginal or no utility in the assessment of urothelial neoplasms. Superficial cells are in contact with the urinary space. They are large, elliptical cells that lie umbrella-like over the smaller intermediate cells. They may be binucleated and have abundant eosinophilic cytoplasm (mills& stacyE 2007).

In the distended bladder, they become flattened and barely discernible. While the presence of these cells is taken as a sign of normaly of the urothelium, one must be aware that they may become detached due to superficial erosion during instrumentation or tissue processing in the prosecting area. Conversely, it is possible to see umbrella cells overlying frank carcinoma. In summary, the presence or absence of superficial cells cannot be used as a determining factor of malignancy (Koss LG, 1969; Newman J, 1989; Fawcett DW et al, 1994).

Lamina Propria:

The lamina propria lies between the mucosal basement membrane and the muscularis propria. It is composed of dense connective tissue containing a rich vascular network, lymphatic channels, sensory nerve endings, and a few elastic fibers. In the deeper aspects of the lamina propria, of the urinary bladder and ureter, the connective tissue is loose, allowing the formation of thick mucosal folds when the viscus is contracted. Its thickness varies with the degree of distention and is generally thinner in the areas of the trigone and bladder neck. In fact, in patients with urinary outflow obstruction (i.e., prostatic hyperplasia), the bladder neck may contain muscularis propria directly beneath the mucosa, with the lamina propria being virtually indiscernible. In the midportion of the lamina propria of the bladder lie intermediate-sized arteries and veins. Wisps of smooth muscle are commonly found in the lamina propria and usually are associated with these vessels (Ro JY et al 1989; Dixon JS, Gosling JA, 1983).

Muscularis Propria:

The muscularis propria is said to be composed of three smooth muscle coats, inner and outer longitudinal layers, and a central circular layer. In fact, these layers can only be identified consistently in the area of the bladder neck. In other areas, the longitudinal and circular layers mix freely and have no definite orientation. In the contracted bladder, the muscle fibers are arranged in relatively coarse bundles that are separated from each other by moderate to abundant connective tissue containing blood vessels, lymphatics, and nerves. Mature adipose tissue may also be present. Very infrequently, one may see nests of paraganglia, usually associated with neural or vascular structures. The cells are arranged in discrete nests or cords and have clear or

granular cytoplasm with round or vesicular nuclei. They should not be confused with invasive carcinoma. Immunohistochemical stains for cytokeratins are negative but for chromogranin are positive. Similar to other layers, the thickness of the muscularis propria will vary from patient to patient, with age, and with the degree of distention. For staging purposes, the muscularis propria has been divided into two segments, superficial and deep (T_{2a} and T_{2b}, respectively). No anatomical landmarks can be used to make this distinction so it must be done by direct visualization on the light microscope. Prior transurethral resection will alter the anatomy of the site and mask normal landmarks, making proper staging difficult if not impossible (Olgac S et al, 2004).

Serosa:

The serosa is composed mainly of peritoneal covering. In the male, the superior surface is covered completely with peritoneum, which extends slightly to the base and is continued behind into the rectovesical pouch. In the female, the superior surface is covered almost entirely with peritoneum, but posteriorly the serosa is reflected onto the uterus to form the vesicouterine pouch. (mills & stacyE, 2007).

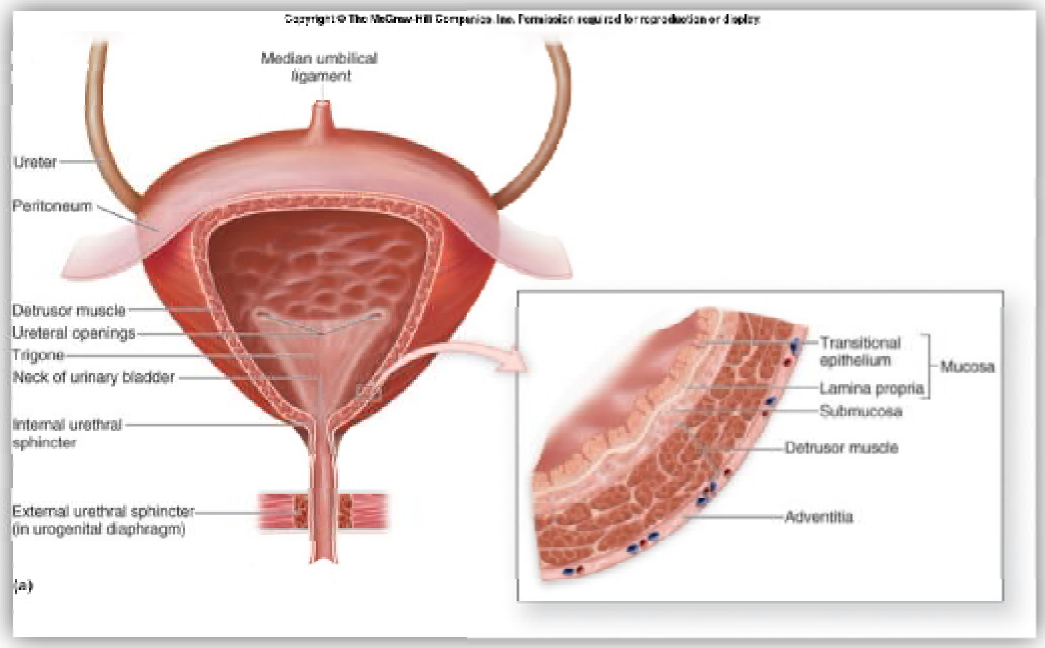


Figure (2-4): histology of urinary bladde

(Clinical Anatomy 2003)

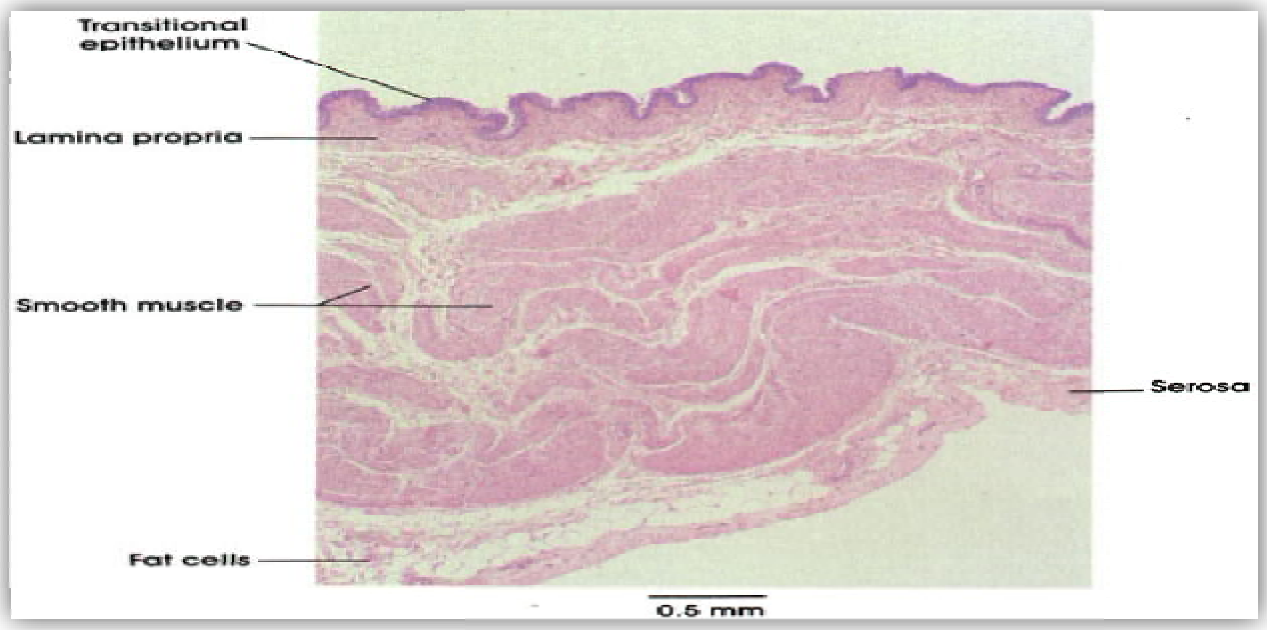


Figure (2-5): Normal urothelium

www.vh.org/adult/provider/anatomy

The urinary bladder carcinoma:

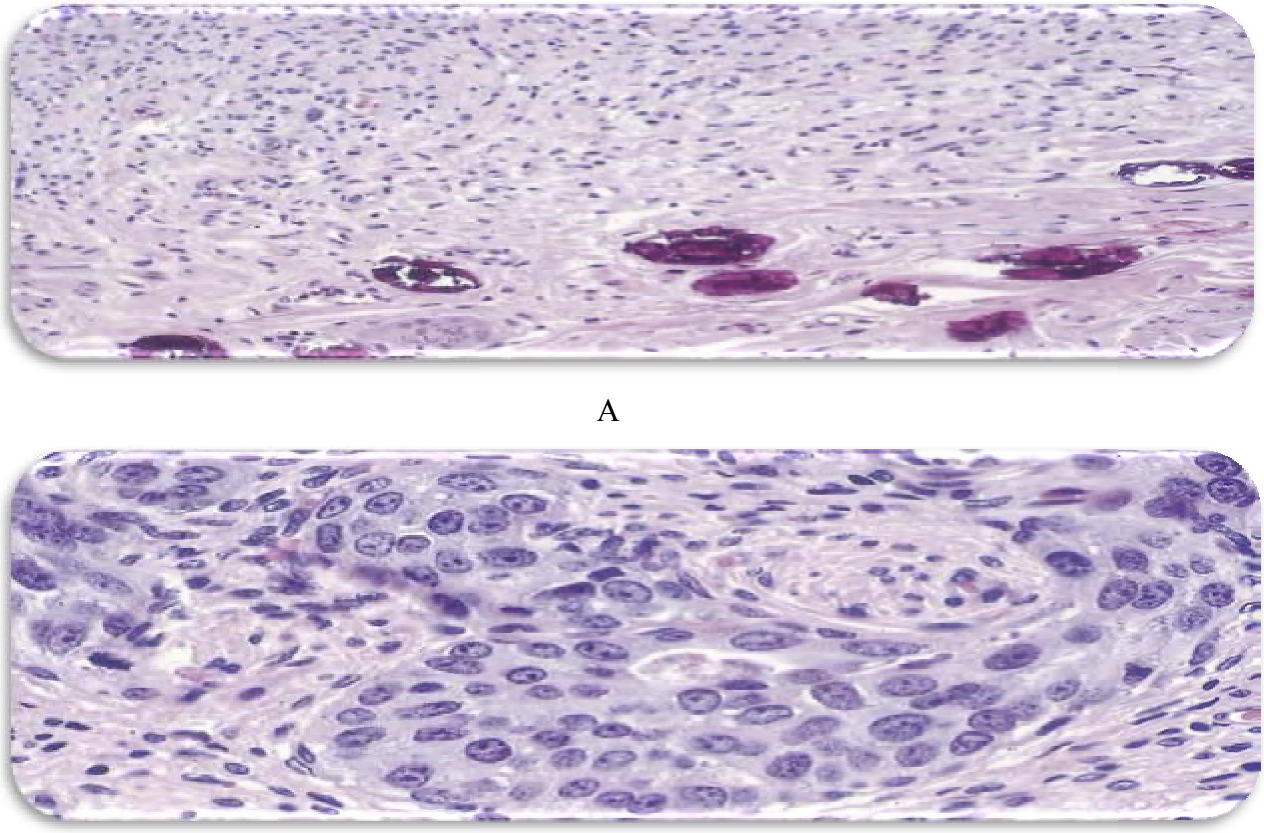
The urinary bladder is the most common site of urinary tract tumors, Tumors are more common in men than in women, Most tumors are microscopically classified as urothelial (transitional cell) neoplasms about 90%, Squamous cell carcinomas 7%, adenocarcinomas 1%, neuroendocrine carcinomas and sarcomas are rare. This distribution is in contrast to schistosoma haematobium endemic areas, such as Egypt and parts of middle east where squamous cell carcinoma is the most form of bladder cancer, Most tumors are malignant, but their aggressiveness and prognosis vary, depending on the clinical stage and microscopic grade and type of each tumor.Tumors are often multifocal and can occur in any part of the urinary tract lined by transitional epithelium, from the renal pelvis to the posterior urethra. Most of the urinary bladder tumors are located on the posterior and lateral walls of the bladder (70%), only about (20%) are located on the region of the trigone and neck , the remaining (10%) are on the bladder dome (Rubin et al, 2008).

Incidence:

Bladder cancer is a common malignant disease. More than 67,000 new cases are diagnosed annually in the United States. The incidence of bladder cancer is higher in the United States and Europe than in many other parts of the world. In the United States, bladder cancer is the 5th most common malignant disease and the 10th most common cause of cancer deaths. The disease is three times more common in men than women (due to smoking), and whites are affected more commonly than blacks (Jamal A etal, 2007).

The high incidence of bladder cancer in men may be explained by environmental and dietary exposures, innate sexual characteristics (e.g., anatomic differences), urination habits, or hormonal factors. Most bladder cancers occur in adults who are more than 50 years of age, and the disease has a male predominance. Cases also can occur in younger individuals, even children, but are still more common in male patients and are usually low grade, with a favorable clinical outcome. The mortality rate for bladder cancer has declined since the 1950s, partly because of improvements in diagnosis and treatment (Hartge P et al, 1990; Fine SW et al, 2005).

Approximately 90% of urinary bladder tumors in the United States are transitional cell type, 7% are squamous, 2% are glandular, and 1% are undifferentiated .This distribution is in contrast to Schistosoma haematobium –endemic areas, such as Egypt and parts of the Middle East, where squamous cell carcinoma is the most common form of bladder cancer see figure (2-6). In S. haematobium infection, the toxins produced by the organism itself or through secondary bacterial infection damage the urothelium, a process that can lead to squamous metaplasia and subsequently to squamous cell carcinoma (although adenocarcinomas and urothelial carcinomas are also observed). The patients usually present at a younger age and with advanced stage (Tawfik HN, 1987; Mostofi FK et al, 1988).



A

B

Figure(2-6): Section of bladder wall from an Egyptian man. **, (A)** Note the presence of Schistosoma eggs, associated with pronounced inflammation. **, (B)** A section from the same bladder showing squamous carcinoma **(Modern surgical pathology 2009)**.

In libya, bladder cancer prevalence accounting for about 8% of the total cancer cases and was ranked the 3rd after breast cancer(10.6%) &colorectal cancer(9.1%) in study on the incidence of cancer in eastern part of Libya conducted in the period between 1991&1995. About 327 cases of genitourinary tract cancer were diagnosed in males out of which 149were in the urinary bladder (45.6%) ,meanwhile 302 cases of genitourinary tract cancers were diagnosed in females only 25 cases were in the urinary bladder (8.3%) (Al-fituri Omran, 1995).

In Benghazi, a total of 67 bladder cancers were diagnosed in Benghazi Cancer Registry (BCR) during 2004 with a huge predominance in men (52 cases, 77,6 % of all cancers in males) over women (15 cases, 22,3%). Transitional cell carcinomas were the most common type of cancer (60% of all microscopically verified cases). The age standardized rate (ASR) was 12.6/100,000 in men and 3.8/100,000 in women, with a remarkable difference with the estimated cancer incidence for Libya by Globocan 2002 especially among males. The difference is probably due to the fact that the Globocan 2002 estimate is based on the average of neighboring countries and that includes Egypt, where the estimated incidence rate in males is the highest in the world (ASR: 37.1 per 100,000), although both Algerian and Tunisian Cancer Registries reported much lower incidence rates (Benghazi Cancer Registry, 2004).

Mortality:

Mortality from bladder cancer is high in the elderly. For instance the ratio of disease related mortality to incidence rates for men and women age 65 to 70 years is 16%and 24% , whereas that for men and women age 80 and older is 40% and 60% .The mortality rate for bladder cancer has declined since the 1950s, partly because of improvements in diagnosis and treatment (cancer in Wisconsin – 1992, 1994).

Regional and national differences:

The incidence of urinary bladder cancer has been reported to be somewhat higher in the North of the United States than in South, the incidence in different countries also differs considerably, with higher rates in Great Britain and the United States than in Japan and Finland . In Hawaii, the incidence is more than twice as high in whites as in those of Japanese descent. These

differences probably reflects combined effects of environmental and hereditary factors (water house J et al, 1982).

Clinical features:

Signs and symptoms:

The type and severity of clinical signs and symptoms of urinary bladder carcinoma depends on the extent and location of the tumour. Most patients with urinary tumours present with at least microscopic hematuria (Messing EM, Vaillancourt A, 1990).

The most common presenting symptom of bladder cancer is painless gross hematuria which occurs in 85% of patients. Subsequent clotting and painful micturition may occur. In case of large tumours bladder capacity may be reduced resulting in frequency. Tumours located at the bladder neck or covering a large area of the bladder may lead to irritative symptoms, i.e. dysuria, urgency and frequency. Similar symptoms may be present in the case of extensive carcinoma in situ. Tumours infiltrating the ureteral orifice may lead to hydronephrosis, which is considered a poor prognostic sign. Rarely, patients with extensive disease present with a palpable pelvic mass or lower extremity oedema. In case of advanced disease weight loss or abdominal or bone pain may be present due to metastases (Haleblian GE et al, 1998).

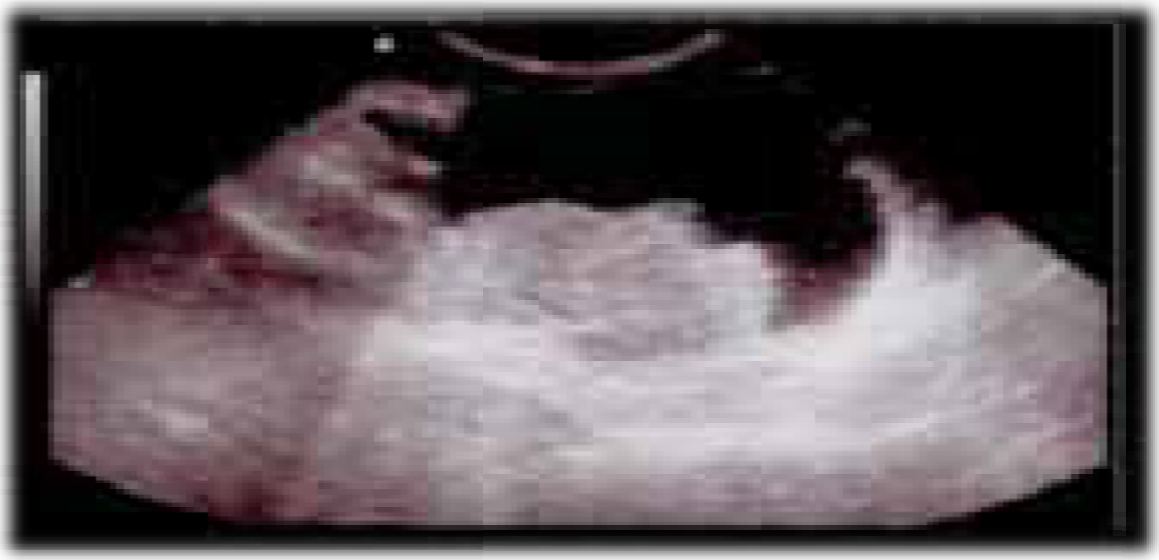
Although diagnosis of a bladder neoplasm may sometimes be suspected on ultrasound or computed tomography scan, it is confirmed on cystoscopy. Histological diagnosis is secured by resecting the tumour deep into the muscular layer of the bladder wall. A fraction of patients with T1 disease may be treated by repeat transurethral resection alone. However, in case of extensive disease most patients are candidates for potentially curative treatment like BCG& Chemotherapy. Upper tract tumours occur in less than 4% of patients with bladder tumours (Anderstrom C etal, 1989).

Imaging:

Various imaging modalities are used not only for detection but also for staging of infiltrating urothelial carcinoma. They include ultrasound, intravenous urography (IVU), computed tomography (CT) and magnetic resonance imaging (MRI). Transabdominal ultrasonography of the bladder is quick, non-invasive, inexpensive and available in most institutions. However, staging accuracy is less than 70% for infiltrating bladder tumours (Denkhaus H et al, 1985).

Sensitivity reaches only 63%, yet with a specificity of 99% . There is a high false negative rate for ultrasound examination because of tumour location, obesity of the patient or postoperative changes. Transurethral ultrasonography may increase accuracy to >95% for T2 and T3 bladder tumours. (Koraitim M et al, 1995; Datta SN et al, 2002).

Endoureteric sonographic evaluation of ureteral and renal pelvic neoplasms is technically feasible. However, as endoluminal sonography is invasive and examiner dependent it is not routinely used. Iliac lymph nodes cannot be assessed reliably on ultrasound (Liu JB et al, 1997).



Figure(2-7): Infiltrative urothelial carcinoma. Ultrasound images of a solid bladder tumour. Bladder (black) of the bladder is poor .with tumour (white) protruding into the lumen. (**Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs 2004)**

Risk factors :

Many factors have been linked to the development of bladder cancer ,including cigarette smoke, chemical exposure, analgesics ,and certain urinary infections. These factors may induce genetic changes in the cells and initiate carcinogenesis.

1.Cigarette smoking:

Tobacco smoking is the major established risk factor of bladder cancer. It is estimated that the risk of bladder cancer attributed to tobacco smoking is 66% for men and 30% for women. The risk of bladder cancer in smokers is 2-6 fold that of non-smokers (Brennan P ET AL, 2000). The risk increases with increasing duration of smoking, and for those with the longest history of smoking (60 years or more) reaches approximately 6 in men and 5 in women. The excess of risk is observed also with increasing intensity of smoking (number of cigarettes per day), reaching maximum of about 3 for those smoking 40 or more cigarettes per day. The increase of risk with the increasing duration and intensity of smoking is similar in both sexes but, some studies indicate higher risk in women than in men at the equivalent level of exposure .The risk of bladder cancer goes down after stopping smoking, and 15 years cessation tends to be approximately that of non-smokers. The decrease of risk after cessation is similar in both sexes (IARC, 2004).

2. Occupational exposure:

Bladder cancer is associated with a number of occupations or occupational exposures. The first such association was observed in 1895 by Rehn, who reported high rates of bladder cancer among men employed in the aniline dye industry. Subsequent research among dyestuffs workers identified the aromatic amines benzidine and 2-naphthylamine, and possibly 1 naphthylamine, as bladder carcinogens . It has been estimated that contact with occupational carcinogens causes up to 25% of all bladder tumours (Dietrich H&Dietrich B, 2001; Pashos CL et al 2002).

3. Arsenic:

Several studies showed that use of drinking water containing chlorination byproducts or contaminated by arsenic may increase risk of bladder cancer . An IARC Monographs Working Group reviewed in 2004 the relevant epidemiological studies concluded that arsenic in drinking-water is carcinogenic to humans (Group 1) and that there is sufficient evidence that itcauses urinary bladder cancer. Key evidence came from ecological studies in Chile and Taiwan (China) where large populations were exposed (Smith AH et al, 1998, IARC, 2004).

4-Phenacetin:

Several epidemiological studies indicate that chronic abuse of analgesics containing phenacetin greatly enhance the risk of developing urothelial cancer of the renal pelvis, ureter and bladder. The relative risk has been estimated in the range of 2.4 to more than 6. Early cases have been reported from Scandanavia, Switzerland and Australia (McCredie M et al ,1982; Mihatsch MJ, Knusli C ,1982) .

5-Medicinal drugs:

The cytostatic agent, cyclophosphamide, has long been associated with the development of leukemia and lymphoma. In addition, treatment with cyclophosphamide has been reported to be associated with an increased risk of squamous cell carcinomas and sarcomas, especially leiomyosarcomas. Similarly, chlornaphazine is associated with the development of bladder cancer (Tanguay C et al, 2003).

6-Coffee:

There is no clear evidence of carcinogenic effect of coffee or caffeine in experimental animals, but some epidemiological studies in humans showed elevated risk in coffee drinkers as compared with non-coffee drinkers. Some studies showed increased risk of bladder cancer caused by coffee drinking only in never smokers, while no increase of risk was observed in ever smokers (Hartge P et al 1983, Woolcott CG et al, 2002).

7-Chronic cystitis and other infections:

Chronic cystitis in the presence of indwelling catheters or calculi is associated with an increased risk for squamous cell carcinoma of the bladder (Kantor et al, 1984). Between 2% and 10% of paraplegics with long-term indwelling catheters develop bladder cancer. Approximately 80% of these are squamous carcinomas. Similarly, schistosoma haematobium cystitis appears to be causally related to the development of bladder cancer, often squamous cell carcinoma (Lucas, 1982). In Egypt, where schistosomiasis is endemic among males, squamous cell carcinoma of the bladder (bilharzial bladder cancer) is the most common malignancy. There is also, however, an increased incidence of transitional cell carcinomas in men with schistosomiasis. Cystitis- induced bladder cancer from all causes is usually associated with sever, long-term infections. The mechanisms of carcinogenesis are not understood but may involve formation of nitrite and N-nitroso compounds in the bladder (Tricker et al, 1989).

Precancerous Lesions:

A precancerous lesion is an acquired tissue change such as actinic keratosis .These precancerous conditions may exhibit varying biologic behavior. Some become malignant tumors only occasionally and only after aprolonged period of time ("facultatively"). Others become malignant tumors often and within a short time ("obligatorily").Several histologic forms may be distinguished according to the specific tissue (Riede &Werner, 2004).

Reactive atypia:

This benign lesion typically results from chronic irritation with stones, medical instrumentation, or acute and chronic inflammation, which is evident in the microscopic slides. The urothelial cells have uniformly enlarged vesicular nuclei and prominent nucleoli. There is no nuclear atypia, hyperchromasia, nor any significant variation in the size and shape of the nuclei (Eble JN et al, 2000; Amin MB et al, 2004; Foster CS, Ross JS, 2004).

Atypia, of unknown significance:

This lesion resembles those with reactive atypia, but also shows some irregularities in the distribution of chromatin, nuclearhyperchromasia and pleomorphism, and enlarged nucleoli. The nuclear changes are still mild but are out of proportion with the chronic inflammation in the underlying stroma (Murphy WM et al, 2004).

Hyperplasia:

Clinical Features:

Rare benign urothelial lesion; it may be seen in the flat mucosa adjacent to low-grade papillary urothelial lesion.

Gross Pathology:

Noncontributory

Histopathology:

Thickened urothelial mucosa without cytologic atypia. Marked thickening, rather than a specific number of cell layers, is needed for the diagnosis of flat hyperplasia.(van Oers JM et al, 2006)

Metaplasia :

Metaplasia refers to a change in morphology of one cell type into another type that is considered aberrant (divergent) for that location. The urothelium frequently undergoes either squamous or glandular metaplasia, presumably as a response to chronic inflammatory stimuli such as urinary tract infection, calculi, diverticulae, or frequent catheterization. Squamous metaplasia, particularly in the area of the trigone, is a common finding in women and is responsive to estrogen production. This type of squamous metaplasia is characterized by abundant intracytoplasmic glycogen and lack of keratinization, making it histologically similar to vaginal or cervical squamous epithelium (Fig. 2-8), and is likely to represent a normal histologic variant unassociated to local injury. (Mills et al, 2004).

Urothelial Dysplasia:

-Clinical

Mean age = 60 years, with male predominance (M:F =3:1). Primary dysplasia is rare, and is a strong risk factorfor the development of urothelial carcinoma in situ and invasive cancer. Usually associated with concurrent or prior history of urothelial carcinoma; a risk factor for recurrence and progression. Presents with irritative and obstructive symptoms and/or hematuria

-Macroscopic

Non-specific findings, erythematous or normal

-Microscopic

The term dysplasia is used to encompass previously designated mild and moderate dysplasia and does not include severe dysplasia. Altered polarity with preservation of superficial cells and cytoplasmic clearing. Cells vary in size and shape .Cells with irregular granular chromatin, irregular nuclear membranes, nuclear crowding, and hyperchromasia. The long axes of nuclei are parallel to the basement membrane Lacks cytoplasmic vacuoles, prominent nucleoli, or atypical mitotic figures Figure (2-8) (Lianng Cheng et al, 2002).

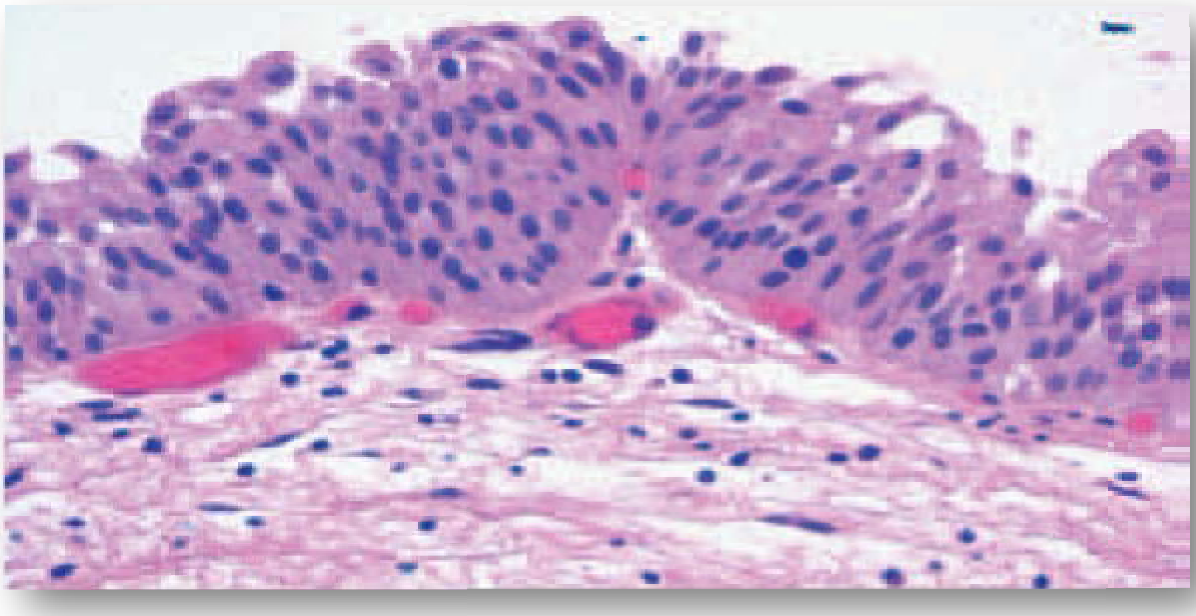


Figure (2-8): Urothelial dysplasia. The urothelium shows nuclear crowding and cytologic atypia. The cells have mildly altered chromatin distribution, slightly enlarged nuclei, inconspicuous nucleoli, and rare mitoses **(Amin MB ,2002)**.

Urothelial Carcinoma In Situ:

-Clinical Features :

Patients are usually in their fifth or sixth decade. Asymptomatic or symptomatic with dysuria, frequency, urgency, or even hematuria. CIS is commonly multifocal and may be diffuse. De novo (primary) CIS accounts for less than 1% to 3% of urothelial neoplasms but is seen with 45% to 65% of invasive urothelial carcinomas.

-Gross Pathology :

Bladder mucosa may be unremarkable or erythematous and edematous

-Histopathology :

Urothelial CIS is a nonpapillary (i.e., flat) lesion in .which the surface epithelium contains cells that are cytologically malignant. The term carcinoma in situ is synonymous with highgrade .intraurothelial neoplasia. Nuclear anaplasia is identical to high-grade papillary urothelial carcinoma. Urothelium may be denuded, diminished in thickness, of normal thickness, or even hyperplastic. There may be complete loss of polarity, marked crowding, and pleomorphism. Nuclei are frequently hyperchromatic and have a coarse or condensed chromatin distribution figure (2-9) (Amling CL, 2001; McKenney JK, 2001; Yin H et al, 2006).

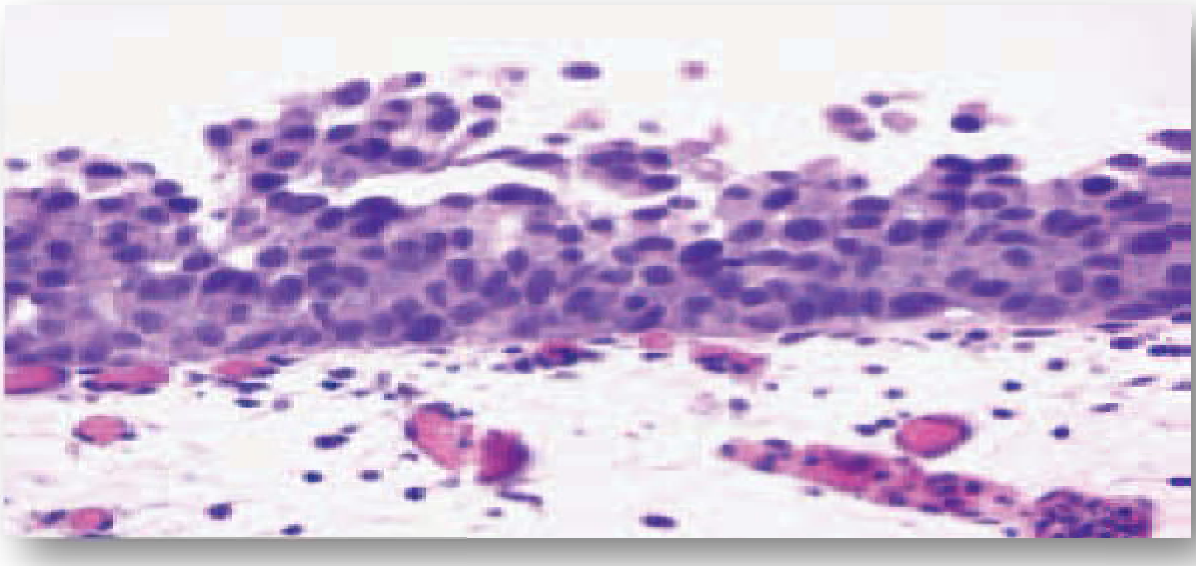


Figure (2-9): Urothelial carcinoma in situ. The entire thickness of the urinary bladder epithelium is replaced by neoplastic cells. There is complete loss of polarity, marked crowding, and pleomorphism. Nuclei are hyperchromatic and have a coarse or condensed chromatin distribution **(Amling CL, 2001; McKenney JK, 2001; Yin H et al, 2006).**

Classification of Malignant Neoplasms of the Bladder:-

Most bladder tumors are epithelial, with 90% classified as urothelial (transitional cell carcinoma) . The terms transitional cell carcinoma and urothelial carcinoma are interchangeable, and we use both terms to denote the primary neoplasms of the bladder. The most important subtypes of epithelial carcinomas of the bladder are adenocarcinomas and squamous cell carcinomas. Most epithelial neoplasms involving the bladder are believed to be of urothelial origin, arising from metaplastic urothelium. The terms transitional cell carcinoma and urothelial carcinoma are used for tumors that have recognizable areas of urothelial differentiation, including tumors that have as a major component areas of glandular, squamous, or other type of differentiation, including small cell and neuroendocrine features. The presence of other components is noted, however, and we often comment on their relative proportion. We reserve the terms adenocarcinoma and squamous carcinoma for tumors that show essentially pure patterns of that designation. (Modern surgical pathology,2009).

Classification of Malignant Neoplasms of the bladder (WHO 2004)

1-Urothelial (transitional cell carcinoma)

-papillary (Papillary urothelial neoplasm of low malignant potential (PUNLMP), low grade urothelial carcinoma,high grade urothelial carcinoma)

-flat carcinoma in situ

-invasive, with or without mixed epithelial components

2-Distintinctive Histological variants

-Micropapillary

-Sarcmatoid /carcinosarcoma

-Lymphoepithelial –like

-Small cell /neuroendocrine

-Giant cell

-Clear cell

-Urothelial carcinoma with trophoblasts

-Plasmacytoid variant

3-Deceptively bland featres

-Tubular

-Microcytic

-Nested

-Endophytic

4-Adenocarcinoma

-Intestinal type (enteric)

- Mucinous

- Signet ring cell

-Clear cell

-hepatoid

5- Squamous Cell Carcinoma

-verrucous

- Basaloid

-Sarcomatoid

6- Urachal Carcinoma

- Mucinous

- Enteric

- Signet ring

7-Mesenchymal Neoplasms

- Leiomyosarcoma

-Rhabdomyosarcoma

-Malignant fibrous histocytoma

-Liposarcoma, angiosarcoma,hemangiopericytoma

8- Other Malignant neoplasms

-Melanoma

-Hematologic

-Carcinoid

-Pheochromocytoma / paraganglioma

• **Papillary urothelial neoplasm of low malignant potential (PUNLMP).**

Papillary Urothelial Neoplasm of Low Malignant Potential (PUNLMP) is a papillary urothelial tumour which resembles the exophytic urothelial papilloma, but shows increased cellular proliferation exceeding the thickness of normal urothelium. The incidence is three cases per 100,000 individuals per year. The male to female ratio is 5:1 and the mean age at diagnosis is 64.6 years .The lateral and posterior walls close to the ureteric orifices are the preferred sites for these tumours (Holmang S et al, 1999).

The papillae of PUNLMP are discrete, slender and non fused and are lined by multilayered urothelium with minimal to absent cytologic atypia. The cell density appears to be increased compare to normal. The polarity is preserved and there is an impression of predominant order with absent to minimal variation in architectural and nuclear features. The nuclei are slightly enlarged compare to normal. The basal layers show palisading and the umbrella celI layer is often preserved. Mitoses are rare and have a basal location.These architectural and cytological features should be evaluated in well oriented, non tangentional cut areas of the neoplasm figure (2-10). The tumours are predominantly diploid. The prognosis for patients with PUNLMP is excellent. Recurrences occur, but at a significantly lower frequency than in noninvasive papillary carcinomas. Rarely, these patients may present with another tumour of higher grade and/or stage, usually years after the initial diagnosis (Malmstrom PU et al, 1987).

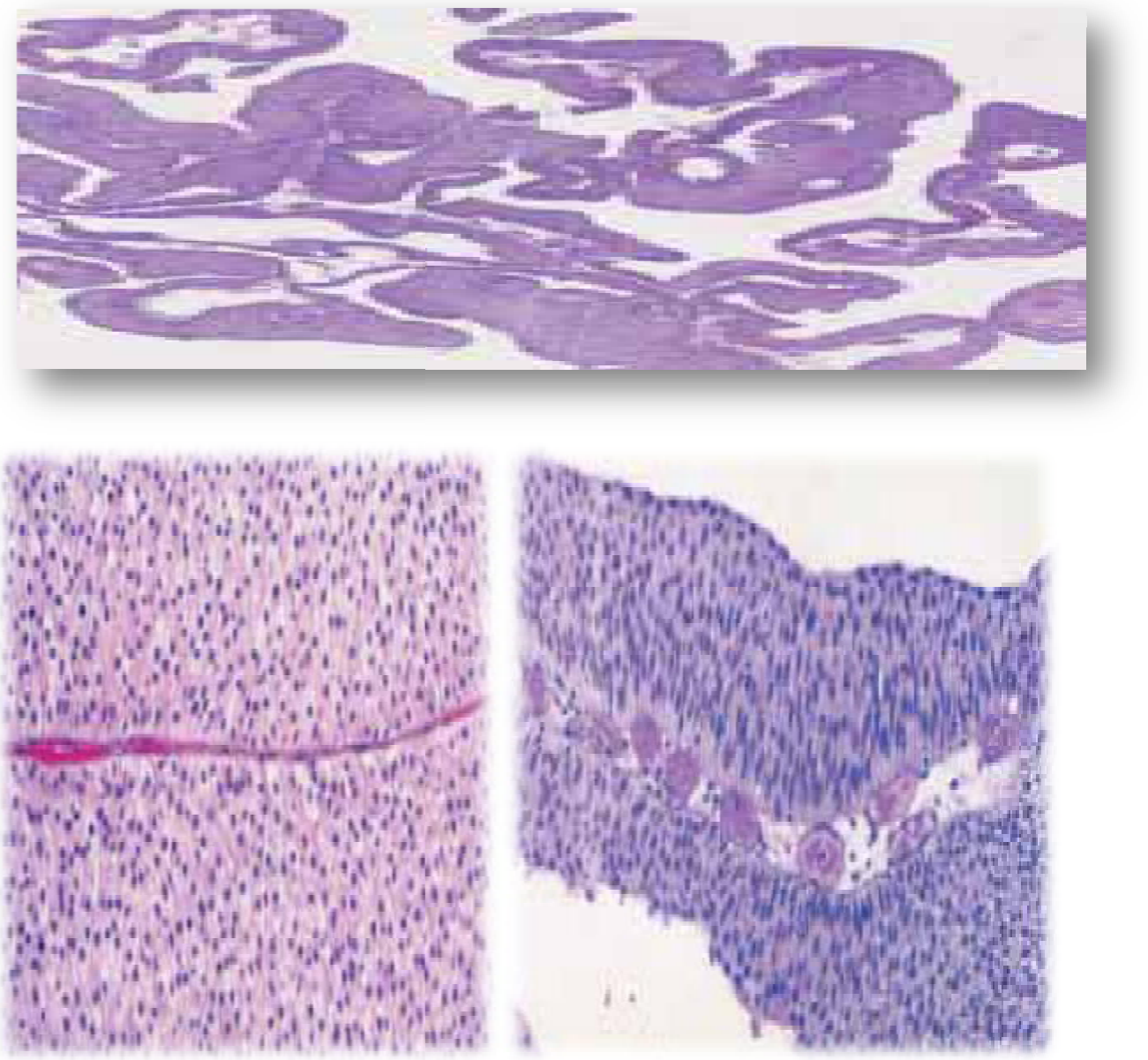


Figure (2-10) : Non-invasive urothelial neoplasm. Papillary urothelial neoplasm of low malignant potential.(X10) **(WHO-urinary& male genital, 2004)**

• **Papillary urothelial carcinoma, low grade.**

A neoplasm of urothelium lining papillary fronds which shows an orderly appearance, but easily recognizable variations in architecture and cytologic features. The incidence is five cases per 100,000 individuals per year. The male-to-female ratio is 2.9:1. The mean age is 69.2 years, .The posterior or lateral walls close to the ureteric orifices is the site of approximately 70% of the cases. (Holmang S et al, 1999).

The tumour is characterized by slender, papillary stalks which show frequent branching and minimal fusion. It shows an orderly appearance with easily recognizable variations in architectural and cytologic features even at scanning power. In contrast to PUNLMP, it is easy to recognize variations in nuclear polarity, size, shape and chromatin pattern. The nuclei are uniformly enlarged with mild differences in shape, contour and chromatin distribution. Nucleoli may be present but inconspicuous. Mitoses are infrequent and may occur at any level but are more frequent basally. The papillary fronds should be evaluated where sectioned lengthwise through the core or perpendicular to the long axis of the papillary frond. If not, there may be a false impression of increased cellularity, loss of polarity and increased mitotic activityIn spite of the overall orderly appearance, there are tumours that show focal high grade areas and in these cases the tumour should be classified as a high grade tumour Figure(2-11).(Pich A et al, 2001).

Expression of cytokeratin p53 immunostaining is intermediate between that of PUNLMP and non-invasive high grade papillary urothelial carcinoma . The tumours are usually diploid. Progression to invasion and cancer death occurs in less than 5% of cases. In contrast, recurrence is common and occurs in 48-71% of the patients (Alsheikh A et al, 2001; Holmang S et al, 2001; Holmang S & Johansson SL, 2002).

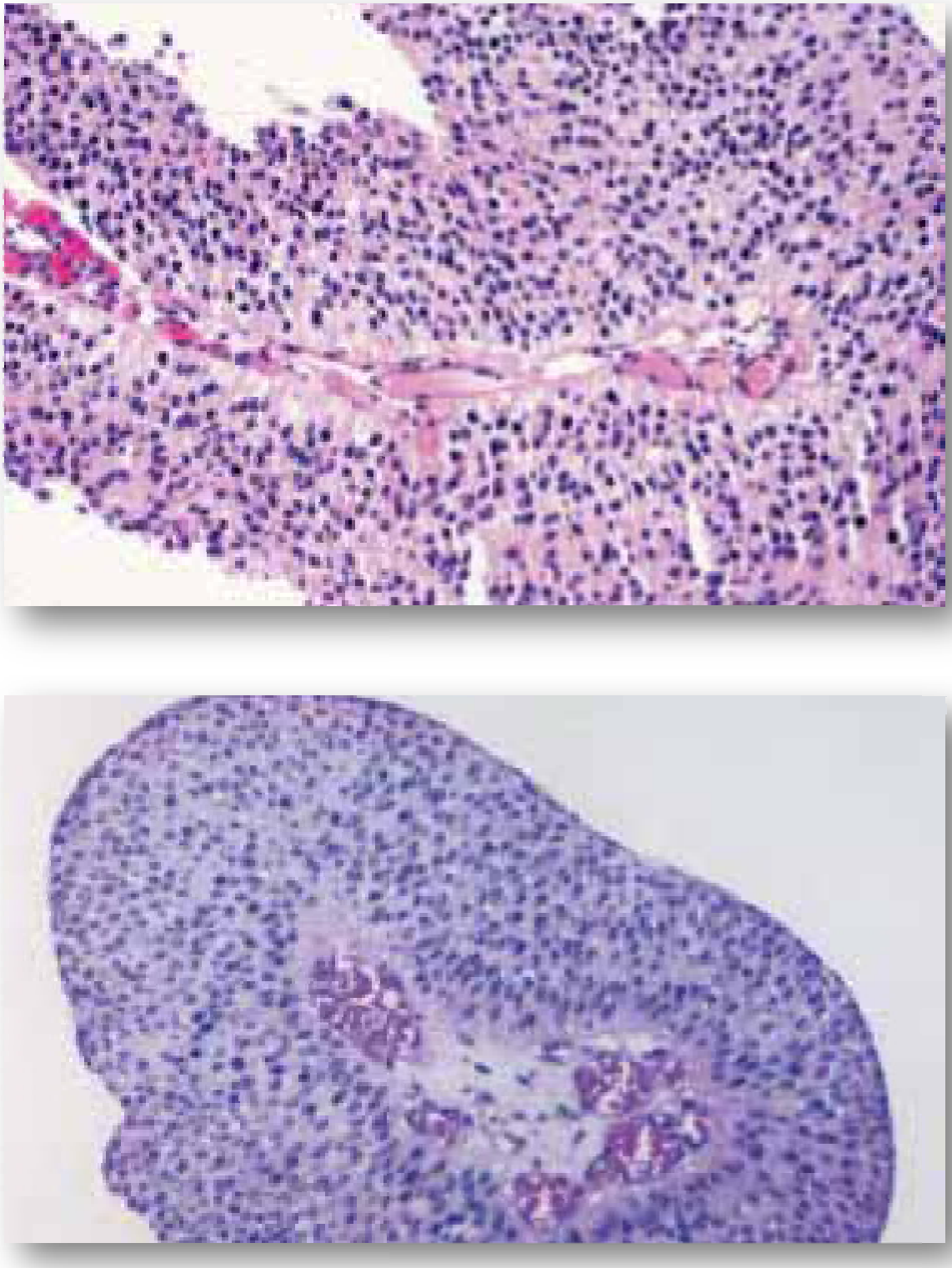


Figure (2-11): Non-invasive low grade urothelial carcinoma **(WHO-urinary& male genital, 2004)**

Papillary urothelial carcinoma, high grade.

A neoplasm of urothelium lining papillary fronds which shows a predominant pattern of disorder with moderate-to-marked architectural and cytologic atypia.The tumour is characterized by a papillary architecture in which the papillae are frequently fused and branching, although some may be delicate. It shows a predominant pattern of disorder with easily recognizable variations in architectural and cytologic features even at scanning power. In contrast to non-invasive low grade papillary urothelial carcinoma, it is easy to recognize more marked variations in nuclear polarity, size, shape and chromatin pattern. The nuclei often show pleomorphism with moderate-to-marked variation in size and irregular chromatin distribution. Nucleoli are prominent Figure (2-12). Mitoses are frequent, may be atypical, and occur at any level, including the surface. The thickness of the urothelium may vary considerably and often with cell dyscohesion. Within this category of these tumours there is a spectrum of atypia, the highest of which show marked and diffuse nuclear pleomorphism. Pathologists have the option of recording the presence or absence of diffuse anaplasia in a comment. The papillary fronds should be evaluated where sectioned lengthwise through the core or perpendicular to the long axis of the papillary frond. Due to the likelihood of associated invasion, including that of papillary cores, these features should be closely looked for. Detection of cytokeratin p53 is more frequent than in low grade tumours . The tumours are usually aneuploid (Desai S et al, 2000; Urist MJ et al, 2002)

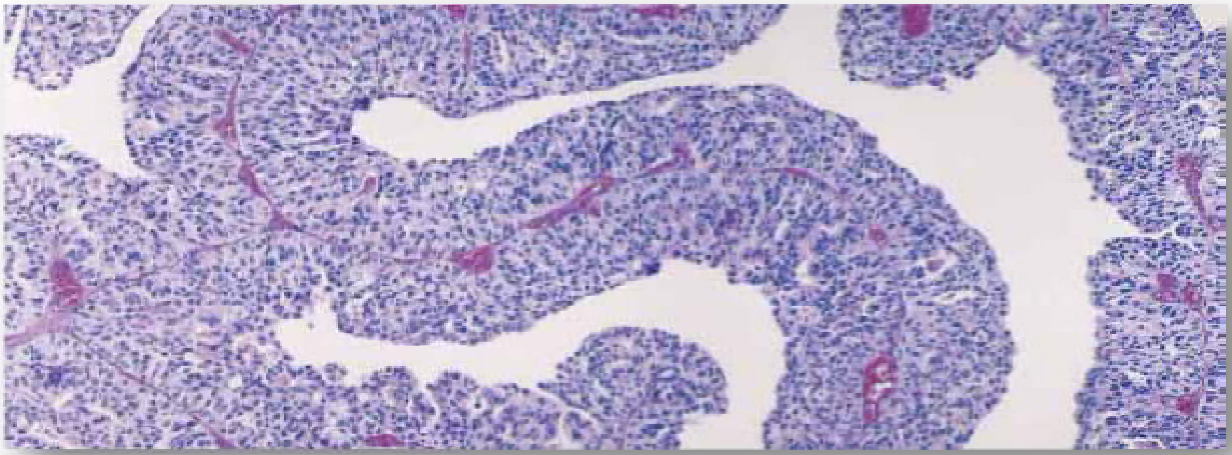


Figure (2-12):Non-invasive urothelial neoplasm. Non-invasive high grade urothelial carcinoma. **(WHO-urinary& male genital, 2004).**

Urothelial Carcinoma Insitu(CIS) :

CIS is a high-grade lesion with severe cytologic abnormalities,similar to those seen in high-grade papillary carcinoma. CIS usually refers to a flat (nonpapillary) lesion, although it can be described within and often is associated with papillary tumors. As its name implies, CIS is a non invasive lesion, and, as with papillary tumors, recognition of early invasion is important.

First described by Melicow as highly atypical epithelium adjacent to invasive bladder cancer, CIS has long been recognized as a dangerous and aggressive form of noninvasive urothelial carcinoma. Melamed and colleagues first recognized that CIS has a high propensity to progress to invasive cancer, and this feature was confirmed by many subsequent studies.Although CIS may occur in the absence of other urothelial tumors (papillary or invasive tumors), it is seen most commonly in association with high-grade papillary tumors or invasive urothelial carcinomas. Clinically, patients with pure CIS either have no symptoms or present with symptoms that resemble cystitis. The cystoscopic and gross appearance of CIS may be subtle, but it is recognizable by urologists and pathologists in most situations. Bladder mucosa involved with CIS shows a hyperemic, velvety appearance.(Zincke H et al, 1985; Mufti GR & Singh M, 1992).

This feature reflects the high degree of angiogenesis observed in response to these tumors and their mucosal friability. An important feature of CIS is its multifocality; involvement of the bladder can be extensive, and CIS can often involve the urethra, prostatic ducts, and ureters. Skinner and coworkers recognized that in bladders where it occurred, CIS was noted at the ureter and urethral margins in a substantial proportion of cases. The multifocality, involvement of urethra (including prostatic urethra and ducts), and involvement of ureteral and urethral margins led some investigators to propose aggressive management for patients with CIS (Koss LG, 1979).

The most common microscopic presentation of CIS is full-thickness replacement of the urothelium by cytologically atypical cells (see Fig. 2-13). The cells have a high nuclear-to-cytoplasmic ratio, moderate to severe nuclear pleomorphism, and an irregular or stippled

chromatin pattern. Mitoses in the middle to upper urothelium are important features. Architecturally, one sees loss of normal urothelial orientation, similar to that noted in high-grade papillary carcinoma. The number of cell layers, or the thickness of the mucosa, can be variable, ranging from hyperplastic to attenuated to fully denuded. This last situation reflects the friability of CIS involving the mucosa and must be recognized as a potential diagnostic pitfall in its presentation. This friability also leads to abundant material for examination in urine by cytology. Several patterns of CIS have been recognized and were well summarized by McKenney and colleagues,89 including large cell CIS, small cell CIS, and CIS with pagetoid spread (Table 2-1) and Although CIS usually is characterized by full-thickness replacement of the mucosa, it now is recognized that cytologic abnormalities can exist without such full-thickness replacement and that the diagnosis of CIS should be based on the presence of cytologic atypia (McKenney JK et al , 2001).

Table (2-1) **Patterns of Carcinoma in Situ** (Am J Surg,2001**)**

1-Large cell CIS with pleomorphism
2-Large cell CIS without pleomorphism
3-Small cell CIS
4-Clinging CIS
5-Cancerization of normal urothelium
6-Pagetoid
7-Undermining or overriding

However, urothelium with less than high-grade cytologic atypia can be seen, and terms such as dysplasia and atypia have been used to describe these types of change. Grading systems similar to those used in papillary carcinomas have been proposed for lower-grade epithelial atypias, but these systems have suffered from lack of reproducibility. In our practice, we report

only the high-grade lesions. The usual situation is in the case of a high-grade lesion that does not involve the full thickness of the mucosa (high-grade dysplasia). (Robertson AJ et al, 1990; Nagy GK et al, 1993; Murphy WM et al, 2000).

An important and well-recognized diagnostic dilemma, particularly at the time of intraoperative frozen section, is the differentiation between CIS and reactive urothelial atypia. Reactive atypia can occur for a variety of reasons and is seen commonly in response to inflammation induced as a result of surgical manipulation, indwelling ureteral stents, or intravesicular treatment. In reactive atypia, nuclear enlargement can be prominent and mitoses may be recognized. However, the nuclear enlargement is generally not accompanied by pleomorphism, and the chromatin pattern is more open than that seen in CIS. The nuclear enlargement in atypia often is accompanied by increased cellular size so that the nuclear-to-cytoplasmic ratio is not as high as in CIS. Although mitoses are common in atypia, they are generally located toward the base of the mucosa and not in the upper levels, as in CIS. Nucleoli can be prominent in reactive atypia. Finally, reactive atypia often is accompanied by visible and pronounced inflammation. When evaluating urothelium, particularly at frozen section for surgical margins, one should note the presence of inflammation. Adiagnosis of CIS in the presence of significant inflammation should be made with great care. Molecular and cellular markers have been advocated to help distinguish atypia from dysplasia, and cytokeratin 20 has been suggested as a possible determinant in this regard, although this method has not yet been widely accepted. CIS is clinically and morphologically distinct from papillary carcinomas and is now recognized to be distinct at the molecular level (Grossman HB et al, 2000; Kunju LP et al, 2005).

Most CIS lesions have Tp53 abnormalities, whereas few papillary carcinomas show Tp53 alterations.Loss of heterozygosity of chromosome 9 and activating mutations in HRAS and FGFR3 genes are commonly observed in papillary tumors and are infrequent in CIS (van Rhijn BW et al 2002; Mitra AP et al ,2006).

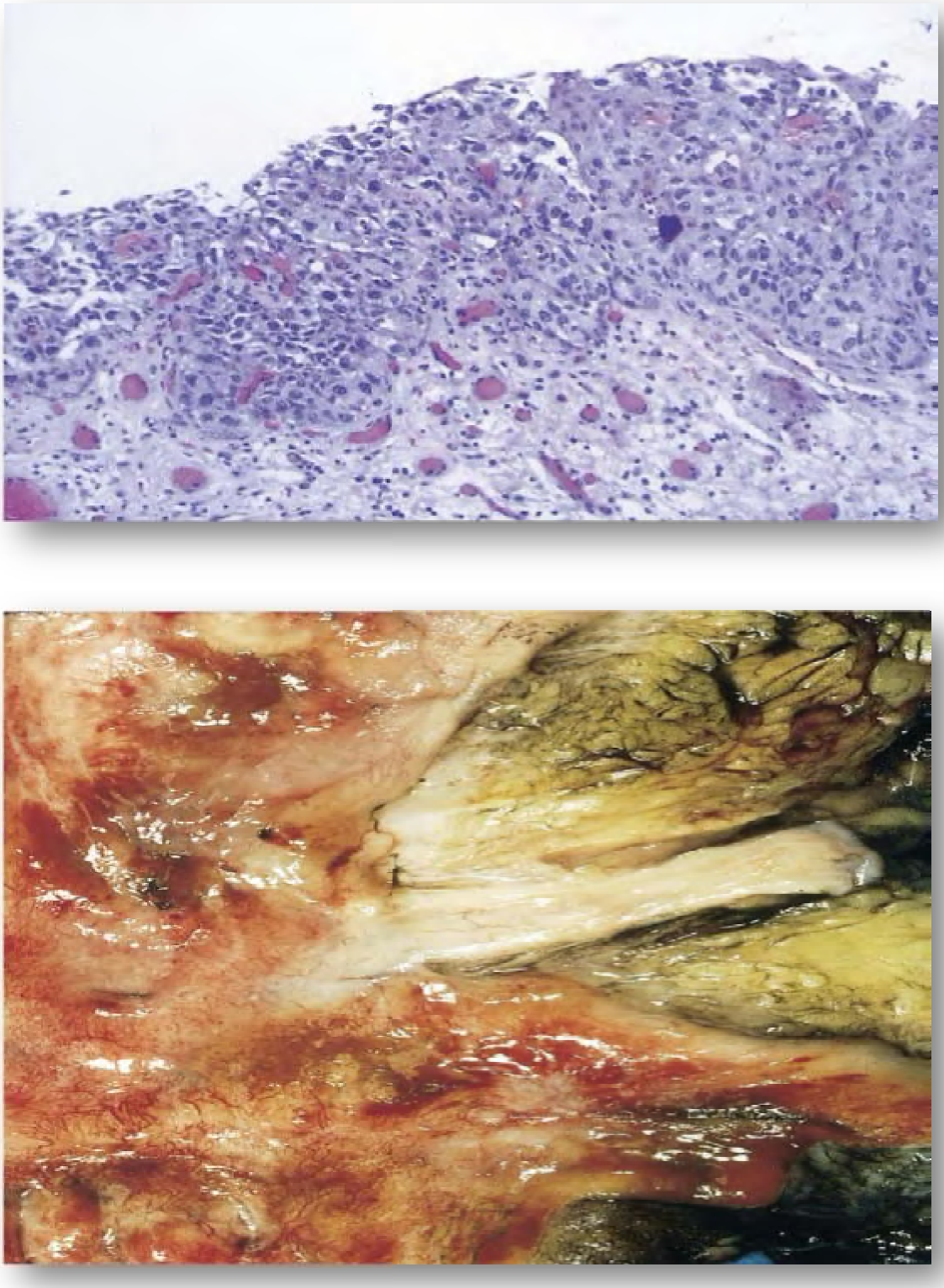
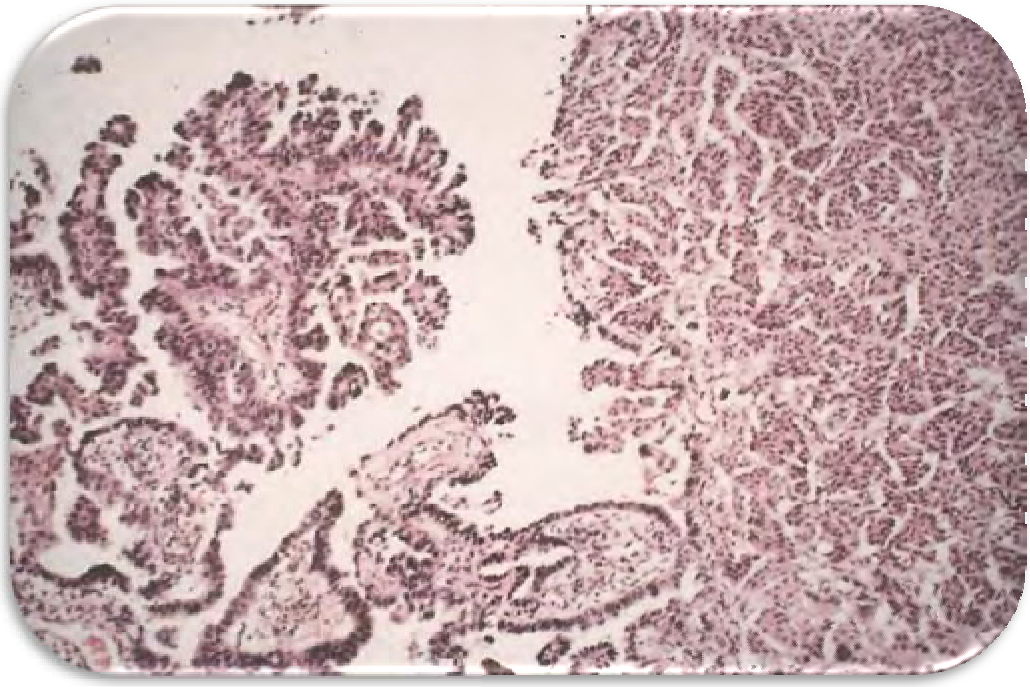


Figure (2-13): Flat urothelial carcinoma in situ (CIS) **(modern surgical Bathology, 2009).**

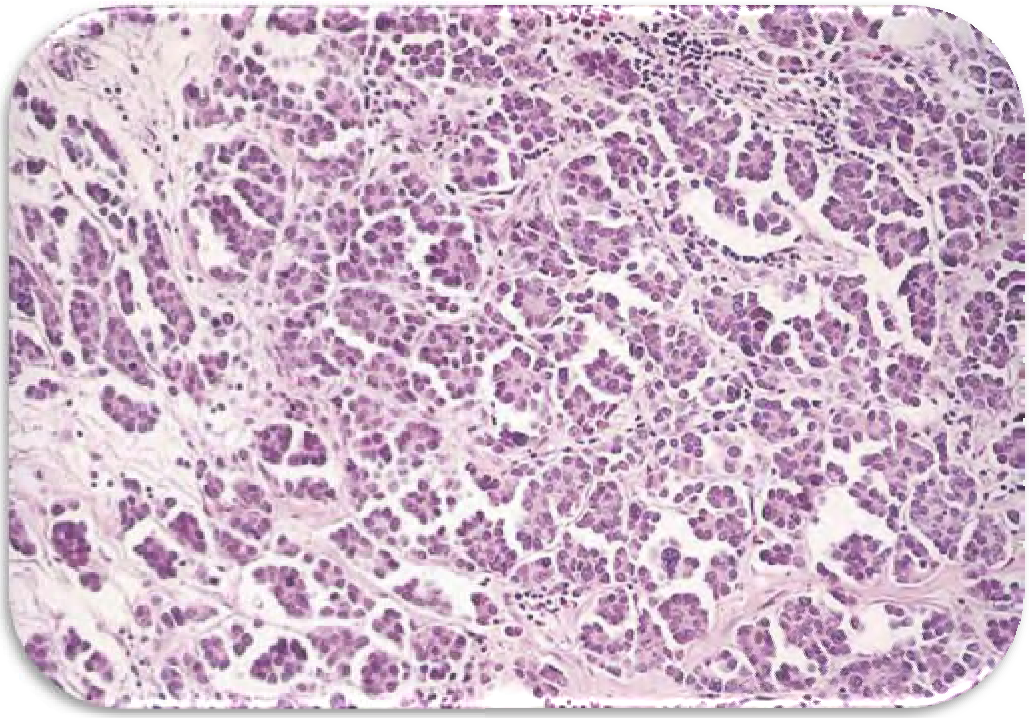
micropapillary transitional cell carcinoma:

One histologic variant of urothelial carcinoma is termed micropapillary transitional cell carcinoma. These tumors have characteristic architecture resembling papillary serouscarcinoma of the ovary. The micropapillary pattern may be focal or extensive, may be part of the surface component, and in some cases is the only pattern seen (Fig. 2-14). The invasive component has well-formed papillary clusters, which can show retraction artifact that mimics vascular invasion. This architectural pattern is retained in metastases from these tumors.(Amin MB et al, 1994).

These tumors can have a deceptively low-grade appearance, with a relatively low nuclear-to-cytoplasmic ratio and nuclei that show minimal to moderate pleomorphism. In contrast to the histologic appearance, this tumor tends to be aggressive; most patients present with disease invading the muscularis propria and many show evidence of metastatic disease at the time of presentation (Samaratunga H et al, 2004).



A



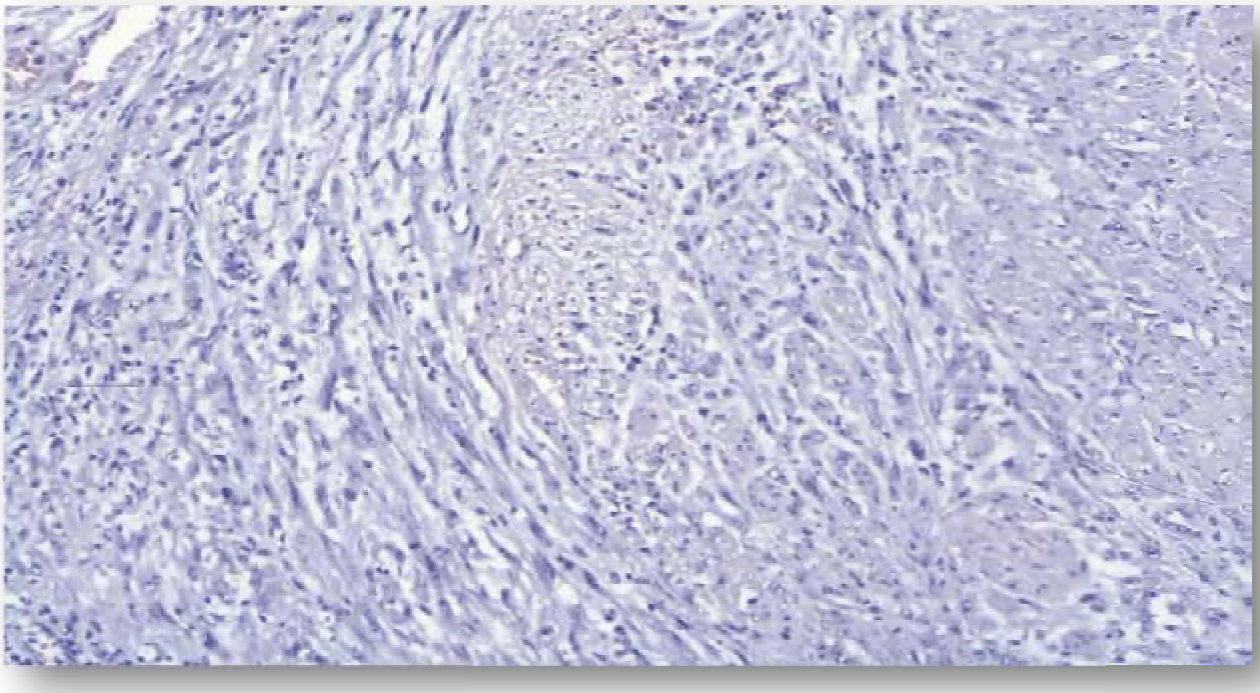
B

(Figure (2-14) : Micropapillary transitional cell carcinoma. **A,** Micropapillary features can be seen as part of the surface component of the tumor. **B,** The invasive component shows well-formed papillary clusters.A prominent feature is retraction artifact that mimics vascular invasion. **(modern surgical Bathology, 2009)**.

carcinosarcoma and sarcomatoid carcinoma:

Sarcomatoid carcinoma is a variant of typical urothelial carcinoma. It is more common than primary sarcomas of the bladder. These tumors have been given a variety of names, including metaplastic carcinoma, spindle cell carcinoma, carcinosarcoma, sarcomatoid carcinoma, and myxoid sarcomatoid carcinoma. The tumors often are associated with urothelial CIS and with elements of more typical urothelial carcinoma or its variants. The tumors frequently manifest as large polypoid masses. Microscopically, the tumors have undifferentiated malignant spindle cells, which are often the most prominent feature (Fig. 2-15). These tumors can have prominent myxoid features and can be associated with heterologous elements, with muscular, cartilaginous, or osseous differentiation, but these features have no particular prognostic significance.The most common form of bladder sarcoma in adults is leiomyosarcoma, which is usually readily recognizable. Sarcomatoid carcinomas should be distinguished from reactive processes, such as

pseudosarcoma, postoperative spindle cell nodules, inflammatory pseudotumors, or a pseudosarcomatous stromal response to urothelial carcinoma. These distinctions are generally not difficult to make because sarcomatoid carcinomas are high-grade pleomorphic tumors and can have prominent myxoid features and a pronounced inflammatory response. The spindle cells of sarcomatoid carcinoma almost always show cytokeratin expression, thereby demonstraing their epithelial origin , and lack expression of muscle antigens. Cytokeratin immune reactivity distinguishes sarcomatoid carcinomas from mesenchymal neoplasms and reactive spindle cell proliferations, although myofibroblasts may occasionally be cytokeratin positive (Young RH et al,1988; Ikegami H et al ,2000).



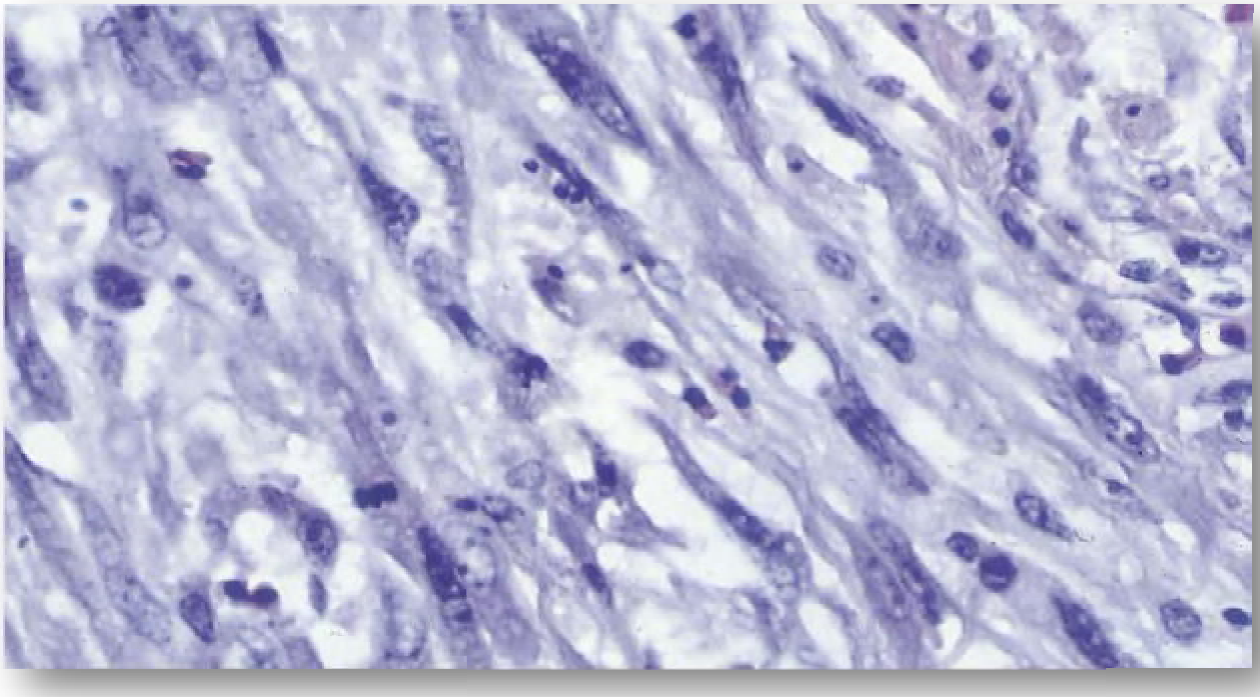
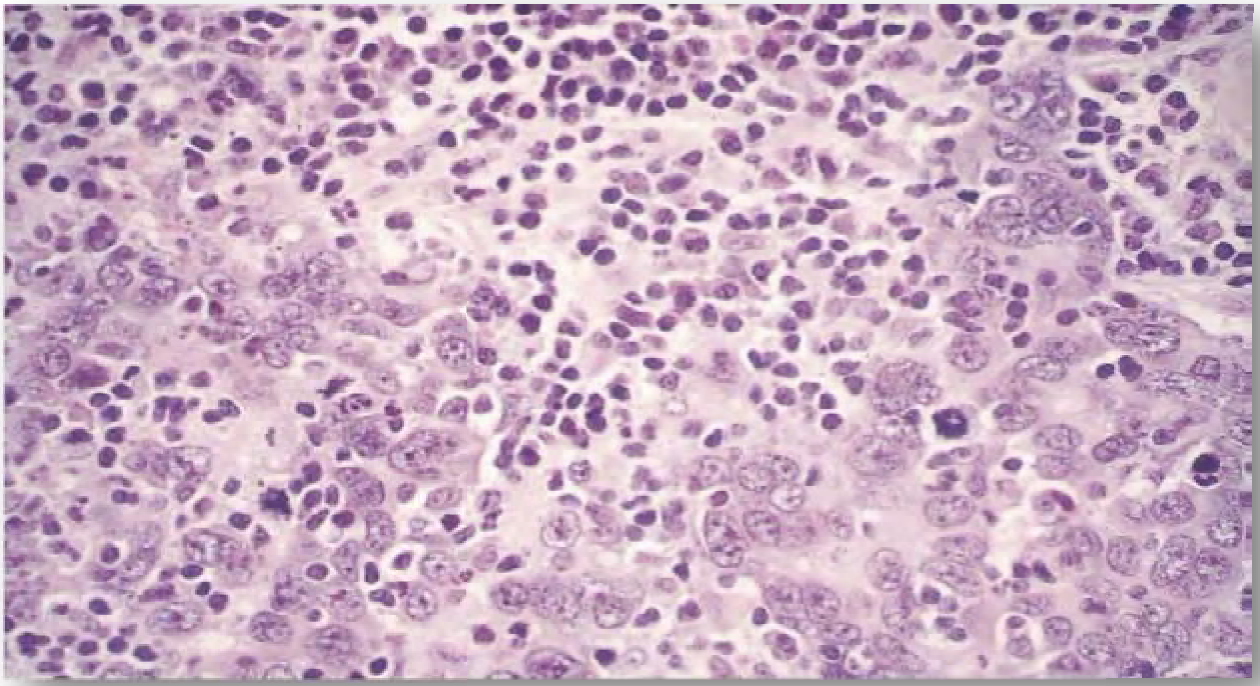


Figure (2-15): Sarcomatoid urothelial carcinoma.**(Modern surgical pathology, 2009)**.

lymphoepithelioma-like carcinoma:

A form of bladder carcinoma that seems to be morphologically and clinically distinct from urothelial carcinoma is lymphoepithelioma-like carcinoma of the bladder. It has a histologic appearance similar to that of lymphoepitheliomas of the nasopharynx, with diffuse sheets of malignant cells having indistinct cytoplasmic borders, large vesicular nuclei with prominent nucleoli, and an associated extensive lymphocytic infiltrate (Fig. 2-16). In contrast to their nasopharyngeal counterpart, lymphoepitheliomas of the bladder are not associated with the Epstein-Barr virus.These tumors are seen either in their pure form or in association with more typical urothelial carcinoma.The differential diagnosis includes lymphoma. Lymphoepitheliomas can be distinguished from lymphomas by their strong expression of cytokeratin . Also included in the differential diagnosis is small cell carcinoma, either primary from the bladder or metastatic.

In general, histologic criteria and the characteristic lymphoid infiltrate can help to make this distinction (Gulley ML et al, 1995; Chen KC et al, 2005).



Figure(2-16): Lymphoepithelioma of the bladder **(Modern surgical pathology2009)**.

Small cell and neuroendocrine cell carcinoma

Small cell carcinoma is a rare bladder tumor that is increasingly described. These tumors have a histologic and immunohistochemical appearance similar to that of small cell carcinomas of the lung and are classified similarly (carcinoid tumors, small cell carcinoma, large cell neuroendocrine carcinoma) (Cheng L et al, 2004; Choong NW et al, 2005). As seen by electron microscopy, they have dense core granules and express neuron-specific enolase, chromogranin, and synaptophysin, findings that confirm their neuroendocrine differentiation. Molecular genetic evidence supports the common clonal origin of bladder small cell carcinoma with urothelial carcinoma. The frequent association of these tumors with typical urothelial carcinoma and other variants supports this view. The tumors are composed of small uniform cells with scant cytoplasm, high nuclear-to-cytoplasmic ratio, finely dispersed nuclear chromatin, absent or inconspicuous nucleoli, and brisk mitotic activity (Fig. 2-17). As seen by immunohistochemical analysis, the tumors have the typical dotlike perinuclear pattern of cytokeratin expression (Abrahams NA et al, 2005; Cheng L et al, 2005).

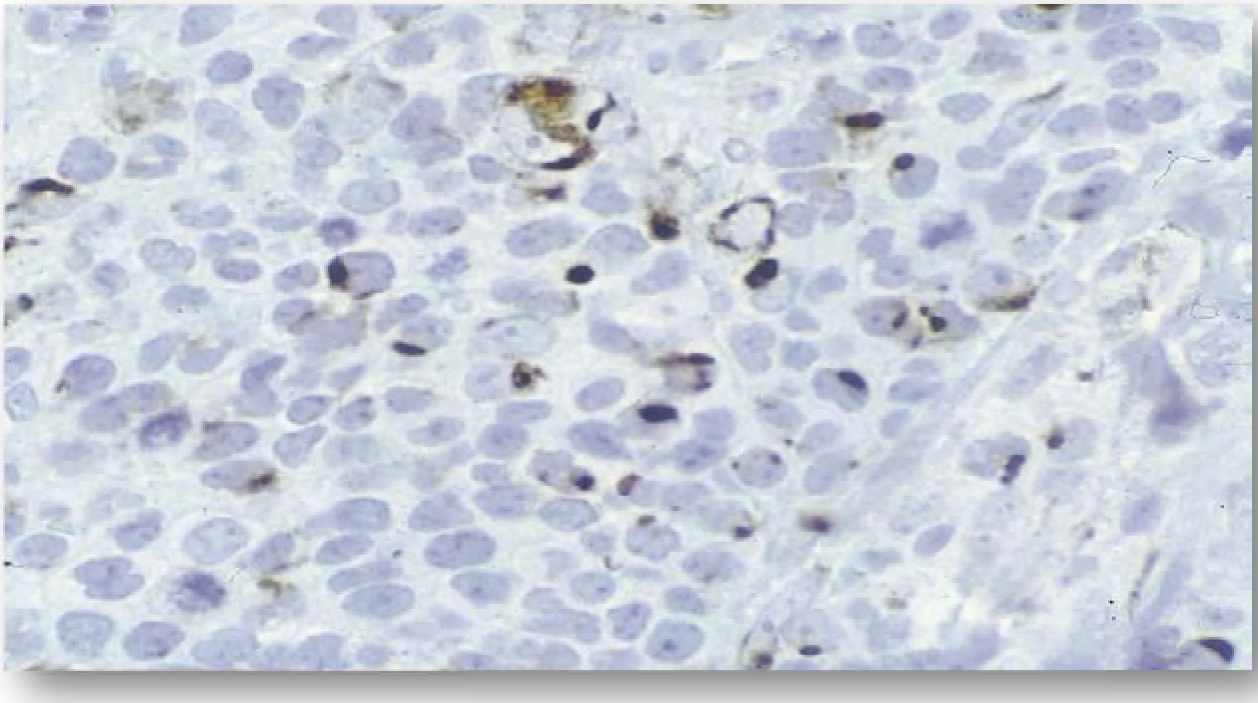


Figure (2-17): Small cell/neuroendocrine carcinoma of the bladder **(Modern surgical pathology 2009).**

deceptively bland variants of urothelial carcinoma:

Urothelial carcinomas have variants that mimic nonneoplastic conditions, such as cystitis cystica, Brunn's nests, nephrogenic adenoma, and inverted papilloma. It is important to be aware of these variants to avoid underdiagnosis, which can be a particular problem in cystoscopic biopsy specimens. Although these variants are uncommon, they can pursue an aggressive biologic course. Four such variants have been described. Transitional cell carcinomas with tubules consist of small nests of cells and tubules, which mimic nephrogenic adenoma (Fig. 2-18).A distinguishing feature is that some of the tubules may be lined by urothelium (Talbert ML et al, 1989; Amin MB et al, 1996).

Microcystic transitional cell carcinoma consists of variably sized cysts within nests of invasive urothelial carcinoma . These cysts may be confused with cystitis cystica, particularly in

superficial biopsy specimens in which the invasive nature may not be readily recognized. Variation in the shape and size of the cysts, identification of nuclear atypia, and the presence of muscularis propria invasion may be helpful in distinguishing microcystic carcinoma from cystitis cystica in superficial biopsy specimens. Microcystic transitional cell carcinoma has also been reported to arise in the urothelium of the renal pelvis (Paz A et al, 1997; Leroy X et al, 2002).

Nested transitional cell carcinoma consists of a packed arrangement of small nests of tumor cells that closely resemble Brunn's nests. These tumors show bland cytologic features that may make the distinction from Brunn's nests particularly difficult, especially in superficial biopsy specimens. Distinguishing features include the presence of larger nests that are irregularly shaped, focal nuclear atypia, stromal reaction, and muscularis propria invasion. This variant is reported to behave in an aggressive fashion despite its bland appearance.These tumors have overexpression of Tp53 and a high proliferation index(Liedberg F et al, 2003; Xiao GQ et al, 2003).

Transitional cell carcinoma with an endophytic growth pattern has broad pushing borders extending into the lamina propria, similar to those seen in verrucous carcinoma . The downward projections have regular contours, but more invasive finger-like extensions may also be seen. Another pattern consists of cords and nests of cells involving the lamina propria and closely resembles inverted papilloma. Nests vary in size and shape and occasionally show microcystic and macrocystic areas. The inverted papilloma-like and broad-front patterns may coexist. When the broad-front pattern is present, the tumor should be considered noninvasive if the basement membrane is intact by light microscopy because the likelihood of metastases is small.Invasion is important to recognize, however, because it increases the risk for metastasis (Young RH et al, 1991; Amin MB et al,1997).

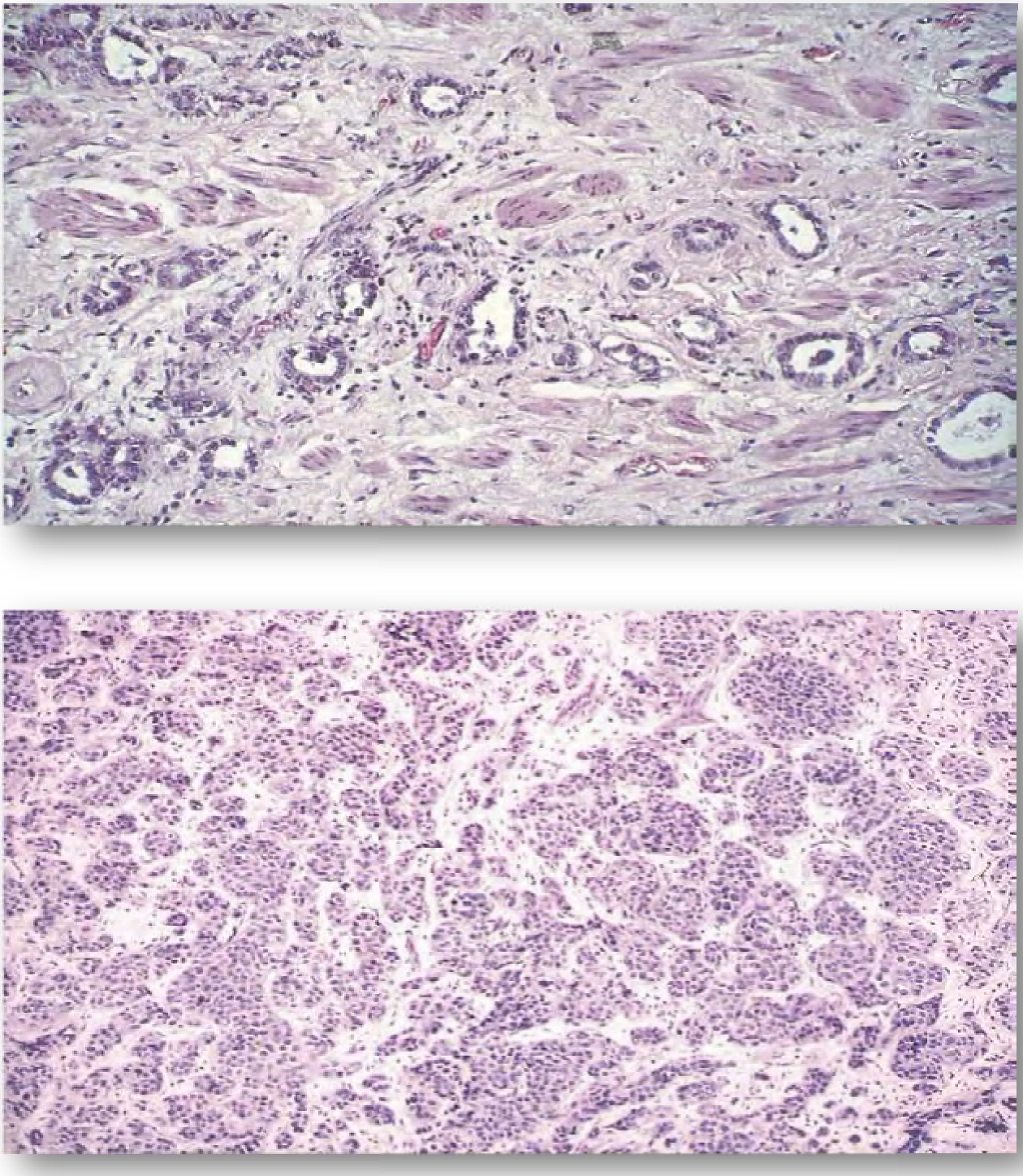


Figure (2-18): Deceptively bland variants of urothelial carcinoma **(Modern surgical pathology2009).**

Adenocarcinoma:

Pure adenocarcinomas of the bladder are relatively rare, constituting approximately 1% to 5% of primary bladder tumors. Risk factors include urothelial metaplasia, most commonly cystitis glandularis with intestinal metaplasia, and exstrophy of the bladder. We have observed adenocarcinoma of the bladder arising in association with glandular metaplasia seen in the surface urothelium (Fig. 2-19). Glandular differentiation can also be seen in cases of recognizable urothelial carcinoma; however, these tumors are classified as urothelial carcinomas with glandular differentiation. Adenocarcinoma arising in the bladder can also be of urachal origin (Zaghloul MS et al, 2006).

Many histologic variants have been described, including adenocarcinoma of no specific type, enteric variants in which the cancer is composed of pseudostratified columnar cells resembling colonic adenocarcinoma, mucinous or colloid carcinoma, signet ring cell carcinoma , clear cell variants, and mixed types in which two or more patterns are found. Although most clear cell variants are considered to be of urothelial origin and are classified as adenocarcinomas, the strong female predominance in these tumors and the absence of endometriosis or of transitional cell carcinoma argue for the presence of a müllerian origin in at least some tumors(Grignon DJ et al,1991; Oliva E et al, 2002).

Ahepatoid variant has been described; these tumors morphologically resemble and have molecular characteristics similar to those of hepatocellular carcinoma, including expression of alpha fetoprotein, α1-antitrypsin, and albumin, and are aggressive with a poor prognosis. Although adenocarcinomas tend to have a poorer prognosis overall than do typical urothelial carcinomas, this is because they generally are found at higher stages at diagnosis. Staging for adenocarcinoma is the same as for typical urothelial carcinomas (Fujii Y et al, 2003).

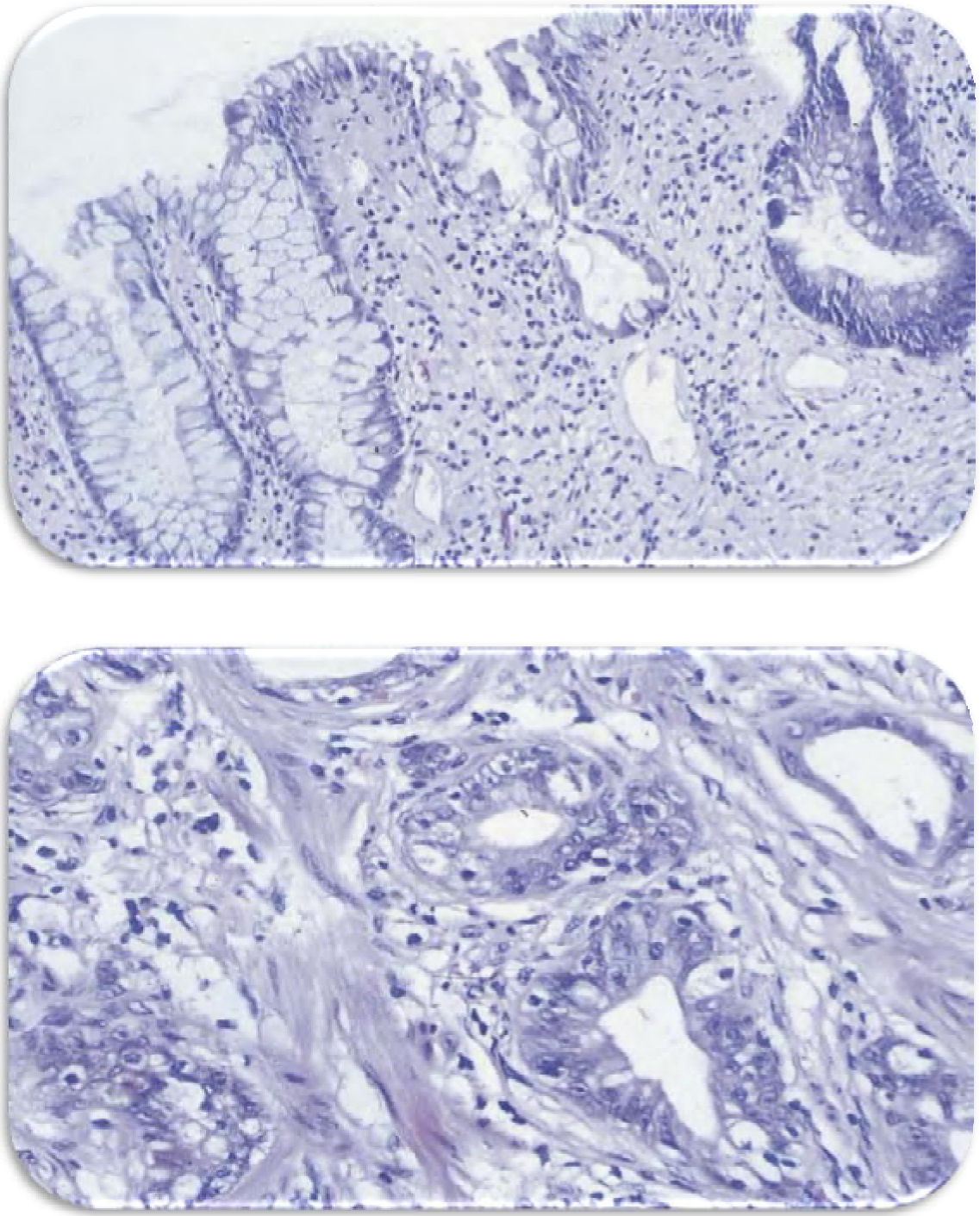


Figure (2-19) : Adenocarcinoma of the bladder arising in association with urothelial intestinal metaplasia and dysplasia **(Modern surgical pathology2009).**

Signet ring cell carcinoma:

Signet ring cell carcinoma is a rare variant of adenocarcinoma. It is seen most commonly in association with well-differentiated forms of adenocarcinoma and with more typical urothelial carcinoma (Fig. 2-20). The pure form of signet ring carcinoma is uncommon. It tends to manifest with diffuse bladder wall involvement, which results in induration and thickening similar to the linitis plastica seen in signet ring cell carcinomas of the stomach. These tumors have a particularly poor prognosis, primarily as a result of advanced stage at presentation. They must be distinguished from metastases and from direct extension of signet ring carcinomas from adjacent sites, in particular rectum, urethra, and prostate. Metastatic lobular carcinoma of the breast also may resemble signet ring cell carcinoma involving the bladder. It is important to distinguish the mixed form of signet ring carcinoma from its pure form because the prognosis is significantly worse for the pure form. These tumors may rarely originate in the urachus.(Grignon DJ et al, 1991).

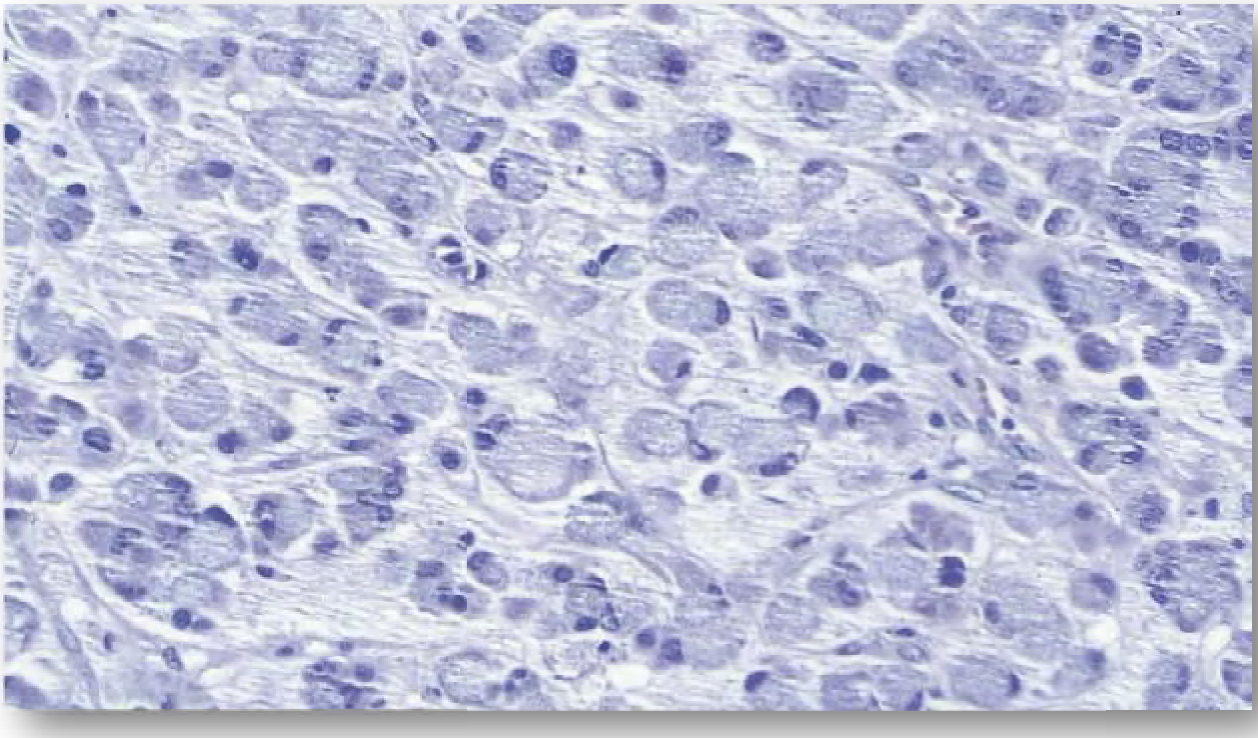


Figure (2-20) : Adenocarcinoma of the bladder with mucinous features.Signet ring cells often are seen in association with better-differentiated forms of adenocarcinoma.**(Modern surgical pathology2009).**

squamous cell carcinoma

In certain parts of the world, particularly where schistosomiasis (S. haematobium) is prevalent, squamous cell carcinoma is the major form of bladder cancer. This type of bladder cancer is seen in the Nile River Valley, and schistosome eggs can be identified in most cases (see Fig. 2-21).In other areas, such as the United States, squamous cell carcinoma comprises 3% to 6% of malignant bladder tumors. In these areas, squamous cell carcinoma is often associated with a history of bladder irritation, such as chronic infection, stones, or the long-term presence of indwelling catheters (such as with paraplegic patients) (El-Bolkainy MN ET AL, 1981).

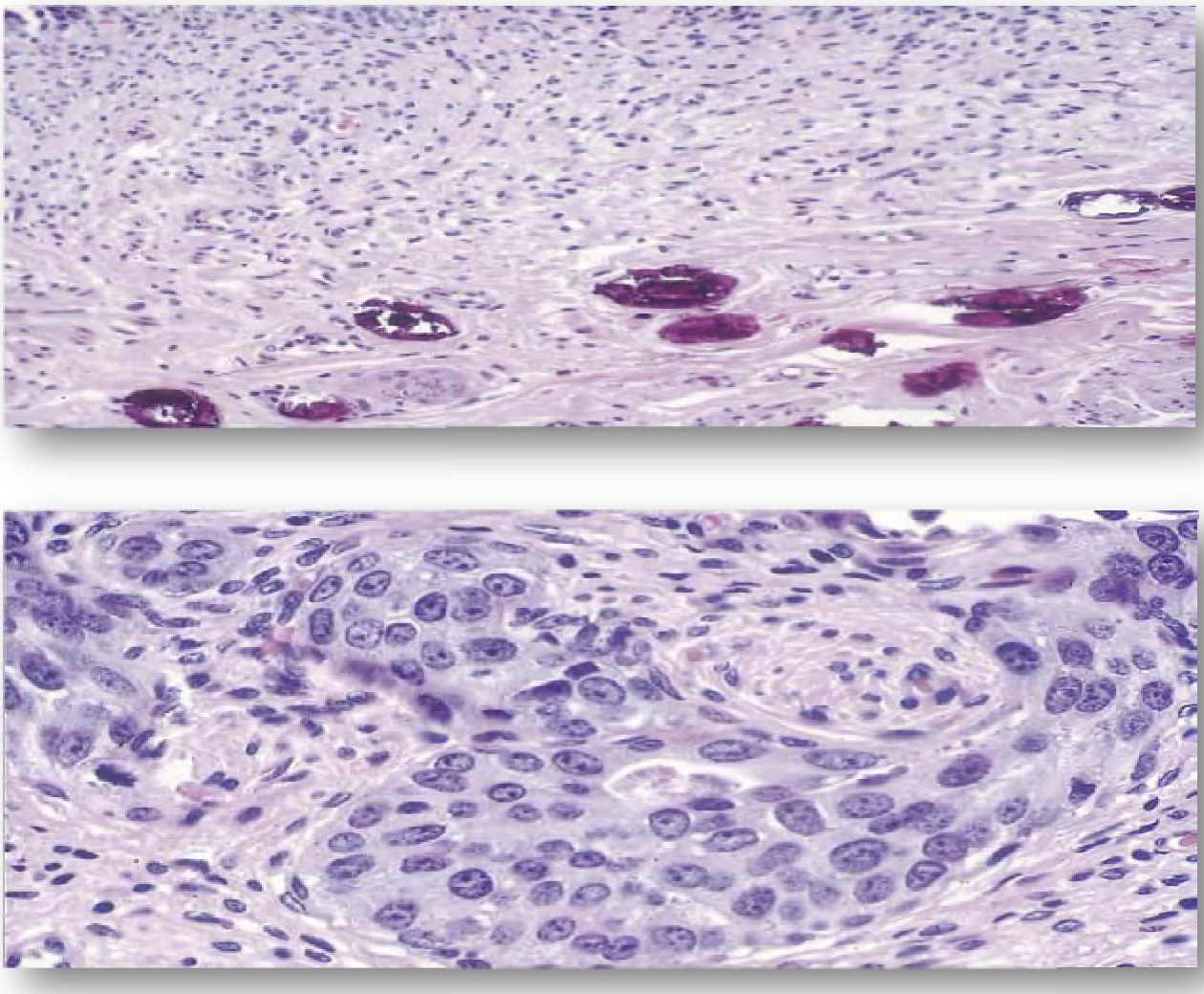
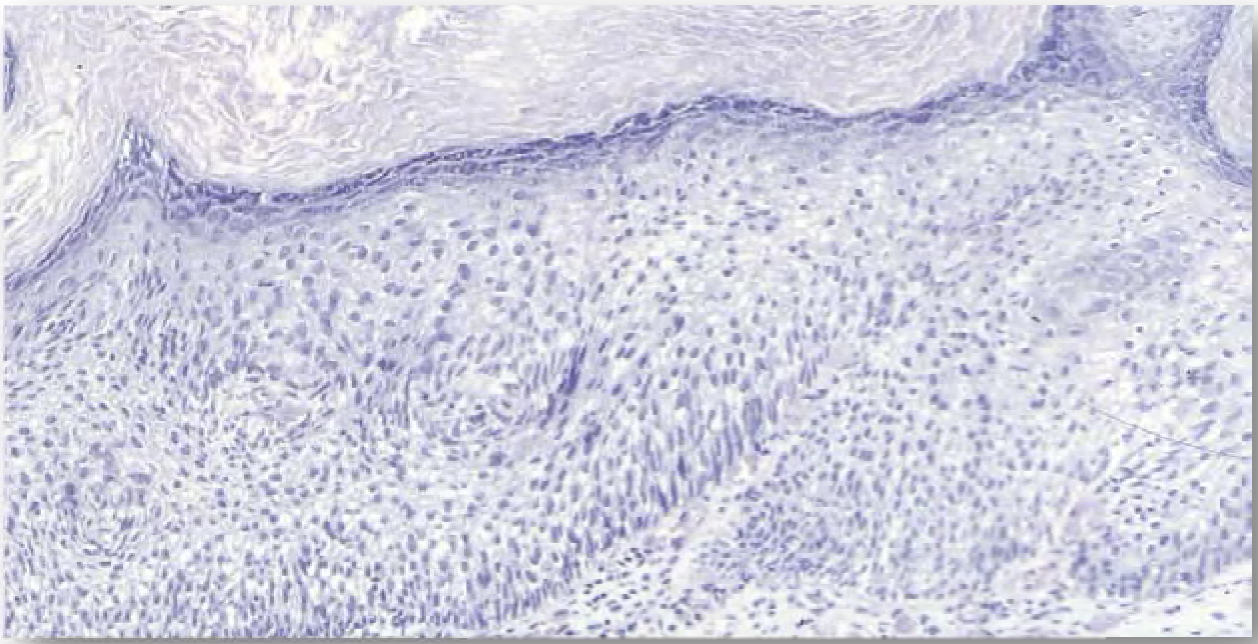


FIGURE (2-21) : Section of bladder wall from an Egyptian man. Note the presence of Schistosoma eggs, associated with pronounced inflammation. A section from the same bladder showing squamous carcinoma **(Modern surgical pathology 2009).**

Tumors arising from bladder diverticula are often squamous cell carcinomas. Rarely, bladder exstrophy can give rise to squamous cell carcinoma.As with adenocarcinoma, squamous cell carcinoma of the bladder is thought to arise from squamous metaplasia of the urothelium, which often is seen in association with this type of bladder cancer. Squamous carcinomas commonly are associated with loss of expression of the cyclin dependent kinase inhibitor p16 through mutation, deletion, or methylation of the p16 promoter.Another possible cause, which has been described only rarely, is infection with human papilloma virus, which can give rise to bladder condylomata and subsequently squamous cell carcinomas, particularly of the verrucous type (Fig. 2-22) (Markl ID, Jones PA, 1998).



Figure(2-22) :Verrucous carcinoma arising in human papillomavirus-associated condyloma. Gross photograph shows the flat and slightly raised areas of squamous metaplasia and condyloma . In the upper part of the photograph, the large verrucous carcinoma is identified **(Modern surgical pathology, 2009).**

urachal carcinoma:

Squamous cell carcinomas should be distinguished from typical urothelial carcinomas with squamous differentiation, which can be seen in a substantial proportion of urothelial carcinomas.

When areas of squamous differentiation are seen, the urothelial carcinoma is considered high grade. Squamous carcinomas are classified as typical or conventional (not otherwise specified), verrucous type, basaloid type, and sarcomatoid. The sarcomatoid variant has spindle cell features. The tumors have squamous cells with keratinization. Intracellular bridges often are seen, particularly in the well-differentiated forms. The verrucous type usually is well differentiated and shows broad pushing borders, whereas the basaloid type resembles basal cell carcinomas of the skin, with smaller, more basophilic cells. The tumors can be graded based on their degree of keratinization and histologic differentiation, although these features have not always been associated with outcome (Tawfik HN, 1987).

Carcinomas arising in the urachus or urachal remnants are generally adenocarcinomas, although they occasionally can have squamous and transitional cell features. Although most adenocarcinomas primary to the bladder arise from urothelium, 20% are of urachal origin.The identification of adenocarcinomas of urachal origin is based on several clinical and pathologic features, the most important of which are location of the tumor in the bladder dome, location centered in the muscular wall rather than in the mucosa, and the absence of intestinal metaplasia or CIS in the surface urothelium (Mostofi FK et al, 1955).

Also helpful are the identification of urachal remnants (Fig. 2-23), a sharp demarcation between tumor and normal urothelium, and the exclusion of metastatic adenocarcinoma from other sites. Most cases occur in individuals in their 40s and 50s, with a mean age of occurrence approximately 10 years younger than for carcinoma arising in urothelium. The most common form of urachal adenocarcinoma is the mucinous type (Grignon DJ et al, 1991).

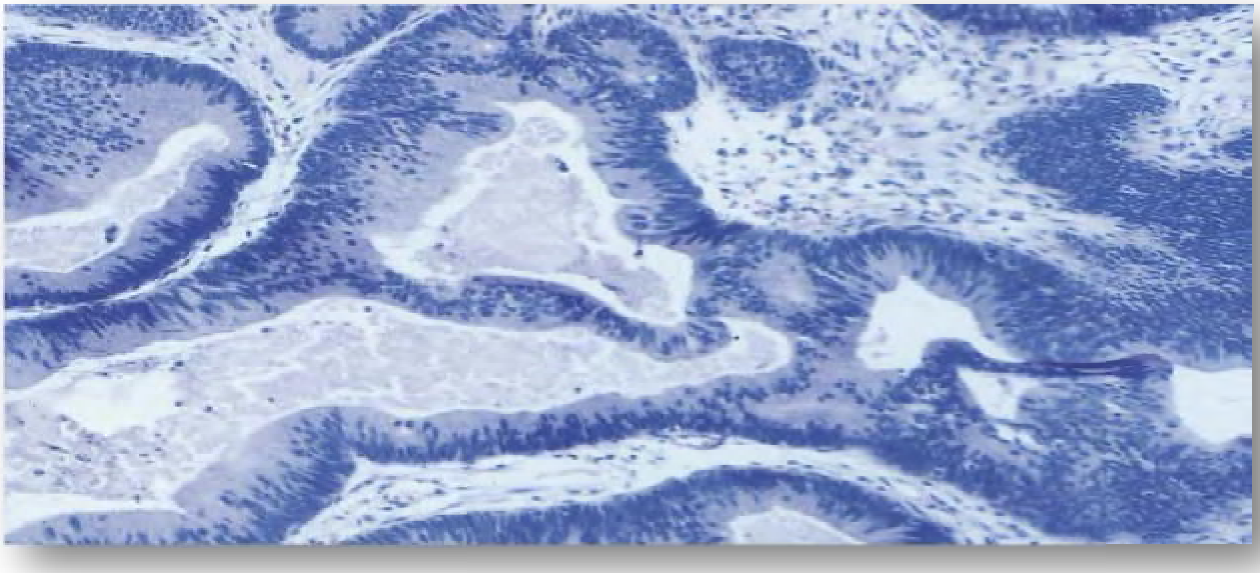


Figure (2-23): Enteric-type urachal carcinoma arising in the dome of the bladder **(Modern surgical pathology, 2009).**

The next most frequent pattern is the enteric type; these tumors closely resemble colonic adenocarcinoma and must be distinguished from colonic adenocarcinomas involving the bladder. A rare subtype of urachal adenocarcinoma has a signet ring pattern.Staging for urachal adenocarcinomas is the same as for urothelial carcinomas.Because these cancers arise in the muscular wall of the bladder, however, and by definition are invading the muscularis propria, a separate staging system has been proposed that evaluates the extent of invasion into the urachus. The importance of distinguishing adenocarcinomas of urachal versus urothelial origin is based on the surgical approach to the tumor. The location of an adenocarcinoma in the dome of the bladder should suggest the possibility of urachal origin, and the urologist should proceed accordingly (Wilson TG et al, 1991).

Sarcomas:

Sarcomas of the bladder are rare and constitute less than 1% of neoplasms arising at that site, although they are the most common malignant tumors involving the bladder in children. Bladder sarcomas show a distinct age distribution: Rhabdomyosarcomas are seen almost exclusively in

children, whereas leiomyosarcomas are the most common soft tissue malignant tumor seen in adults In children, rhabdomyosarcomas are most commonly of the embryonal type and have the typical histologic features of small round blue cells with scant cytoplasm. Gross examination shows polypoid masses extending into the bladder lumen, the so-called botryoid appearance (Russo P et al, 1992).

Rhabdoid or strap cells may be seen, but this finding is not necessary for the diagnosis. Immunohistochemical analysis should be performed on these cases to distinguish them from lymphomas and other tumors. Rhabdomyosarcomas typically express desmin, myogenin, and the myogenic differentiation protein MyoD1, at least focally, and the embryonal type occasionally expresses the p30/32MIC-2 oncoprotein. In adults, rhabdomyosarcomas may have alveolar, pleomorphic, or rarely embryonal patterns. Rhabdomyosarcomas of the bladder traditionally have had a poor prognosis (Fellinger EJ et al, 1991; Triche TJ et al, 1991).

Distinguishing leiomyosarcomas from other spindle cell lesions of the bladder is crucial.Most spindle cell tumors of the bladder that are clearly malignant are epithelial (sarcomatoid carcinomas) and not sarcomas. These cases can be distinguished by immunohistochemical analysis; sarcomatoid carcinomas express keratin at least focally but usually lack expression of muscle antigens. Differentiating leiomyosarcomas from sarcomatoid carcinomas is important because sarcomatoid carcinomas have a much worse prognosis (Jones EC & Young RH, 1994).

Many different malignant mesenchymal neoplasms have been reported arising in the bladder, including malignant fibrous histiocytoma, osteosarcoma, liposarcoma, angiosarcoma, hemangiopericytoma, chondrosarcoma, and alveolar soft part sarcoma (Amin MB et al, 2006).

Metastatic Tumors and Secondary extention:

Secondaries to the bladder are rare and most frequently a late event and are almost always associated with disseminated disease. most mtastatic lesions are secondary to direct extension from tumors of the prostate , lower intestinal tract, and female genital tract. Common primary

tumors that metastasize to the bladder include breast, colon, and kidney carcinomas and malignant melanoma (Helpap B et al, 2004).

Often multiple tumors, typically located in the submucosa , focal ulceration of the urothelium overlying the mass may be seen .the presence of a poorly differentiated tumor sparing the urothelial mucosa should raise suspicion of a possible metastasis. Gland formation may also be seen in metastatic prostate carcinoma (Gattuso et al, 2010).

Grading Papillary Tumors:

Papillary neoplasms are the most common tumors of the bladder, comprising 70% to 80% of all bladder tumors. Papillary tumors tend to recur locally and infrequently become invasive and metastasize. It was recognized by Virchow that papillary tumors of the bladder can exist for many years "and yet no trace of any cancerous infiltration of the base of the growth existed but the tumor was quite simply a papillary one, a benignant formation." However, some bladder tumors act in an aggressive fashion, and investigators have long recognized that the microscopic appearance of a wide variety of tumors correlates with their behavior; this observation has led to classification schemes based on tumor grade Table 2-2). The goal of these schemes is to predict the behavior of tumors (Virchow R, 1978; Busch C et al, 2001; Fujii Y et al, 2003).

In the case of urothelial neoplasms, grading systems are applied to papillary neoplasms. Although all are not restricted to noninvasive neoplasms, grading schemes have the greatest utility in the noninvasive tumors. The first widely used grading scheme was developed by Broders in 1922. This system was based on the degree of differentiation in the lesion and to some extent all other systems are related to it. Many systems and variations on systems have been proposed since then All these systems aim to predict behavior, in particular the risk for local recurrence and development of invasive disease. Although an attempt has been made here to compare the different grading systems directly, the overlap is by no means complete. For example, based on high nuclear grade, some tumors classified as grade 2 in the World Health Organization (WHO) system may be classified as high grade in the WHO/International Society of Urological Pathology (ISUP) system (Bostwick DG & Mikuz G, 2002).

In general, four main grades of papillary neoplasm can be defined, as distinguished by their histologic characteristics. Table (2-3) These grades are important to recognize because they form the basis for treatment and management decisions. Figure (2- 24) The rare, benign papillomas are composed of cells that are arranged in delicate papillary fronds with a central fibrovascular core, with generally no more than seven to eight cell layers Grade 1/low malignant potential (LMP) tumors are composed of essentially normal-appearing urothelium but with a thickness greater than seven or eight cell layers . These tumors are called grade 1 carcinomas in many grading schemes, but it now is recognized that although these tumors may recur, they only rarely progress to invasive or higher-grade lesions. Invasion virtually always is preceded by evidence of increasing grade (or CIS). Recognizing the low propensity of these tumors to progress (either in grade or stage), the ISUP suggested the term papillary neoplasms of LMP (Epstein JI et al ,1998; Cheng L et al, 1999 ; Neumann RM et al, 1999).

Grade 2/low-grade tumors have clear evidence of cytologic atypia, including increased nuclear-to-cytoplasmic ratio, loss of cellular polarity, and infrequent mitoses . Grade 3 and 4/high-grade tumors are clearly cytologically atypical, with features approaching or similar toCIS, including increased nuclear-to-cytoplasmic ratio, loss of normal architectural orientation, and moderate to frequent mitoses. These tumors have a definite propensity for invasion, may have concurrent CIS, and must be followed closely and aggressively. Grade must be reported in all cases of noninvasive papillary tumors. Although less important for invasive tumors, it is still common practice to report grade. The choice of a grading system should be made in conjunction with the urologist and oncologist and should be consistent within an institution. It is helpful to specify which grading system is used or at least to indicate the denominator (Skinner DG, 1980; Busch C et al, 2001).

When reporting grade, the pathologist should list the range of grades seen or the highest grade because management is based on the highest grade of the tumor. The most commonly used grading systems are WHO and Bergkvist, although the WHO/ISUP classification is gaining popularity. The 1998 WHO/ISUP recommended system initially caused some controversy, but more recent studies positively linked the classification to clinical outcome and it has now been

endorsed by the Armed Forces Institute of Pathology and the WHO. Once a tumor has invaded the bladder wall, stage of disease, including depth of invasion, extension to adjacent structures, and lymph node involvement are the most important classic prognostic parameters Although some studies showed that grade remains an important prognostic feature of invasive tumors, more recent studies showed that grade is not an independent predictor of long-term outcome (Stein JP et al, 2001; Eble JN et al, 2004; Murphy WM et al, 2004).

When a tumor has invaded the bladder wall, it almost always is high grade, thus making grading virtually moot for invasive tumors. The impact of specific genetic alterations on the behavior of bladder tumors in general and papillary noninvasive tumors in particular is becoming increasingly clear The presence of Tp53 or Rb alterations is now known to be a risk factor for progression to invasive disease.Studies showed that superficial (Ta/T1) papillary tumors with alterations in Tp53 or Rb tumor suppressor gene products have a higher rate of progression than tumors with no detectable alterations in either Tp53 or Rb. Tumors with alterations in both gene products have the highest rate of progression. Future grading systems no doubt will combine morphology with molecular assessment and will thereby greatly facilitate reproducibility and specific management decisions for patients with papillary neoplasms (Grossman HB et al 1998; Mitra AP et al, 2006).

Table (2-2) : different grading systems of urothelial carcinoma

Ash 1940	Mostofi 1960	Bergkvist 1965	WHO 1973	Murphy	WHO/Isup 1998	WHO/Isup 2004
I.	papilloma	0	papilloma	Papilloma	Papilloma	Lmp
II.	Grade 1	Grade 1	Grade 1	Low grade	Lmp	Grade 1
III.	Grade2	Grade2	Grade2		Low grade	Grade2
IV.	Grade3	Grade3,4	Grade3	High grade	High grade	Grade3

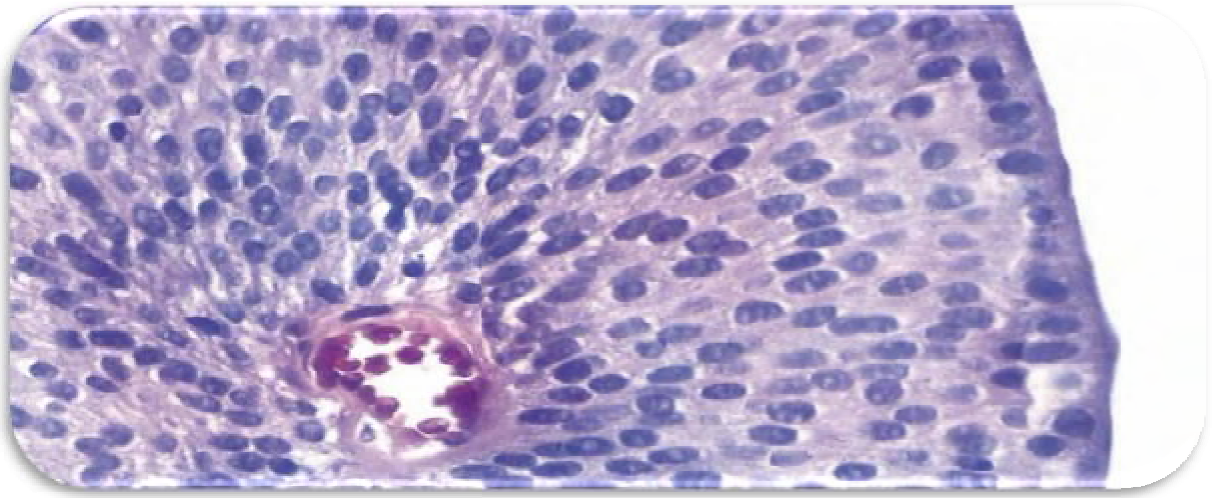
Isup, international society of urological pathology; Lmp , low malignant potential ;WHO, world health organization.

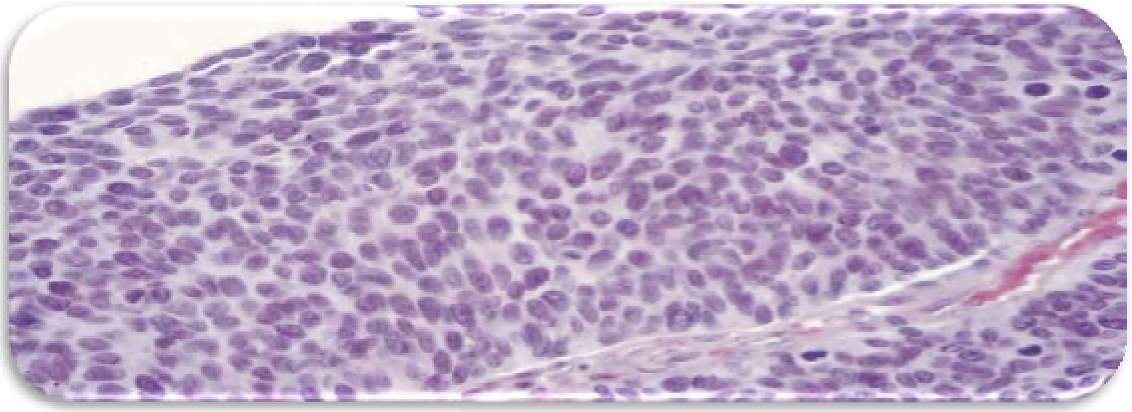
Table (2-3): Histologic features and invasive potential of papillary urothelial neoplasms.

Grade	**Features**	**Risk for Progression/ Invasion**
Papilloma	Normal urothelial cytology Cellular polarity maintained No/rare mitoses Intact umbrella cell layer Seven to eight cell layers	Least
Grade 1/LMP	Normal urothelial cytology Cellular polarity maintained No/rare mitoses	Low
Grade2/ low grade	Umbrella cell layer present or absent Increased cellular density Greater than eight cell layers Moderate urothelial atypia Increased nuclear-to-cytoplasmic ratio Mild to moderate nuclear pleomorphism Loss of cellular polarity Infrequent mitoses Loss of umbrella cell layer Variable cell layer thickness	Moderate

Grade3/ High grade	Marked urothelial atypia Increased nuclear-to cytoplasmic ratio Moderate to marked nuclear pleomorphism Loss of cellular polarity Mitoses easily seen, particularly in upper layers Loss of umbrella cell layer Variable cell layer thickness Dyscohesion Presence of necrosis	**High**

A





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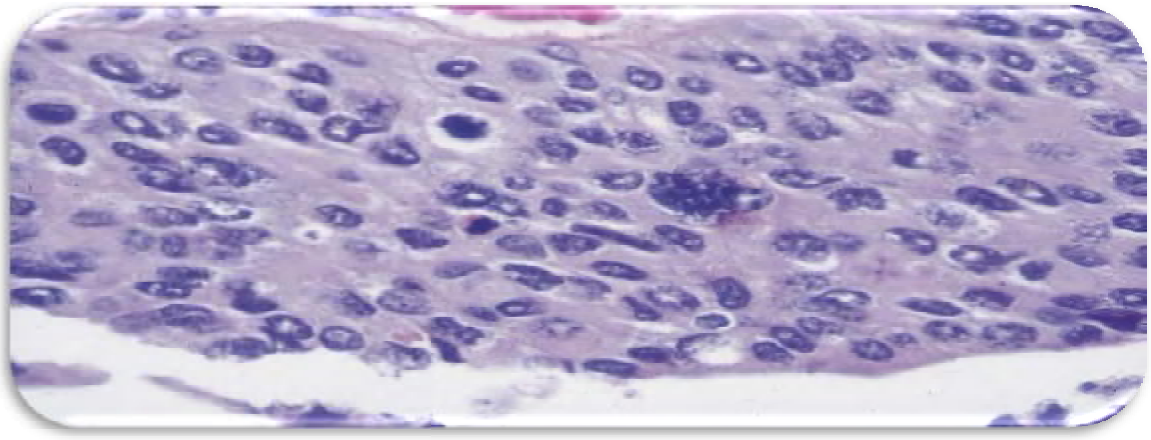


C

Figure (2-24) papillary carcinoma of urinary bladder.

(A) grade 1. (B) grade 2. (C) grade 3. (H&E X40)

(Modern surgical pathology, 2009)

Molecular Staging of Bladder Cancer

Standard methods of bladder cancer assessment are based on histologic grade and stage of the tumor. Although these criteria can provide reliable and reproducible information about populations of patients, they are unable to specify risk for progression or response to treatment for an individual patient. Enormous advances have been made in understanding the molecular basis of bladder tumorigenesis and progression. Bladder cancer is characterized by specific molecular defects , and this information is being translated to develop methods to assess cancer at the cellular and molecular levels. This approach is leading to the development of a new wave of therapeutic interventions targeted at specific disease mechanisms. It is now clear that genes and proteins that exert regulatory control on the cell cycle have important roles in the progression of bladder and other cancers. Attention has focused on genes and proteins that belong to the class of tumor suppressors, in particular p53 .Alterations in Tp53 is among the most common genetic defect in human tumors (Cordon-Cardo C, 1995; Cote RJ&Chatterjee SJ, 1999).

This a protein play an important role in cell cycle regulation, specifically by inhibiting the G1/S transition.154 Several studies showed that Tp53 alteration, as determined by immunohistochemical techniques, is an important predictor of bladder cancer progression (Fig. 2-25). Increased mutant Tp53 immunoreactivity has been found in higher-grade and higher-stage bladder cancers and is associated with disease progression and decreased survival (Esrig D et al, 1994).

It had been shown that Tp53 nuclear accumulation is associated with an increase risk of recurrence in patients with invasive bladder cancer. This association was most pronounced in patients with organ-confined tumors, in which Tp53 nuclear accumulation was found to be the only independent predictor of disease progression when compared with tumor stage and grade. On the basis of evidence from many independent studies, Tp53 assessment seems to be a reliable and consistent prognostic marker for bladder cancer progression (Lipponen PK, 1993; Esrig D et al, 1994).

The ultimate goal of studying molecular determinants of bladder cancer outcome is to define risk and response for the individual patient. Bladder cancer is a model of modern cancer management, in which advances in pathology, surgery, oncology, and basic science have converged to produce the possibility of a more rational, biologically based approach. Understanding of molecular pathways is leading to the development of an increasing array of non traditional treatment possibilities. The evaluation and integration of pathologic and molecular parameters that have a direct bearing on the management of individual patients are clearly the purviews of the surgical pathologist (Deisseroth AB & DeVita VT Jr, 1995).

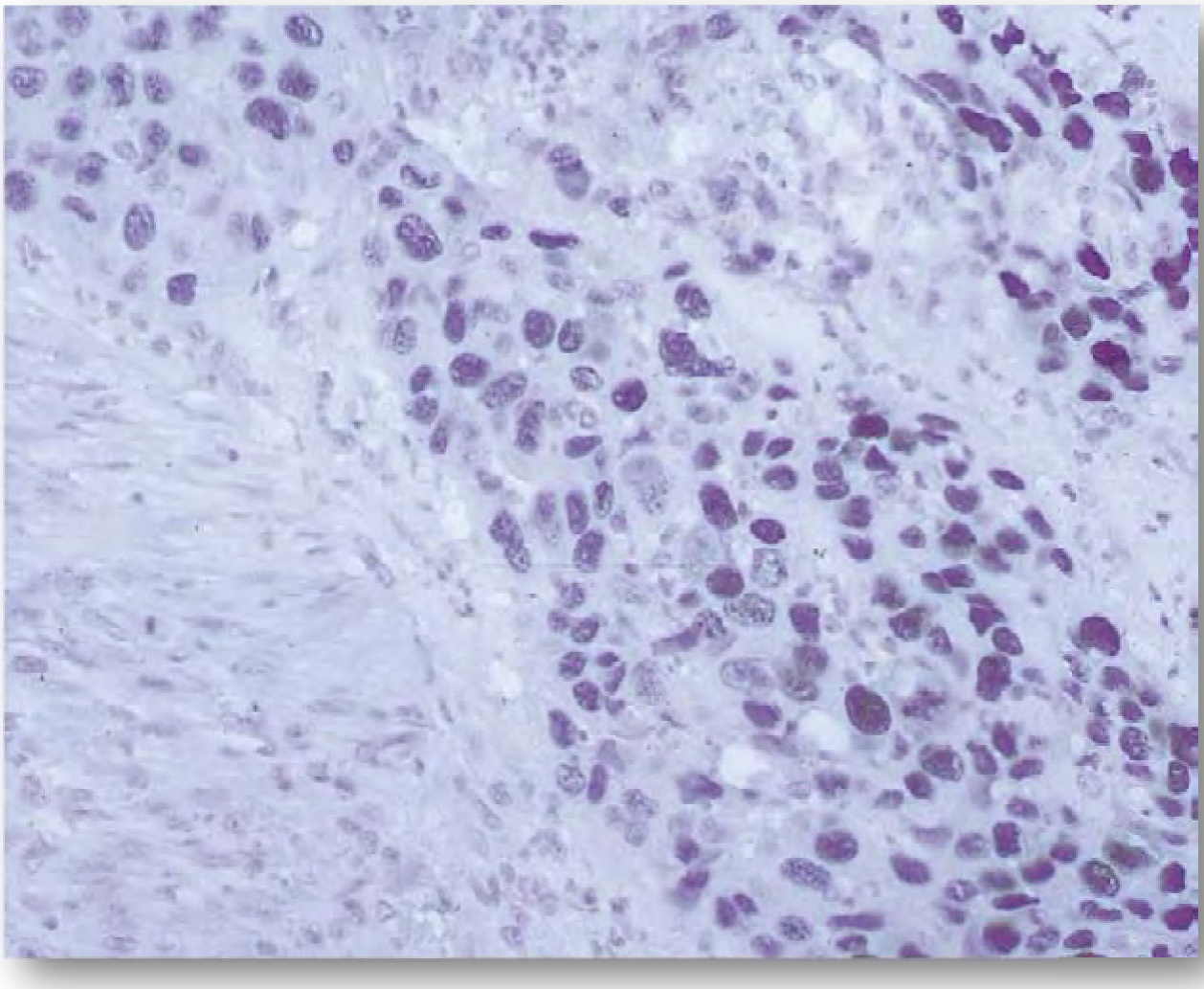


Figure (2-25): Invasive urothelial carcinoma showing nuclear immunoreactivity for the p53 protein, using monoclonal antibody clone 1801. This is indicative of alterations in the p53 gene.**(Modern surgical pathology 2009)**

Staging of urothelial carcinoma:

Currently, pathologic staging of bladder cancer should be performed according to the 2009 AJCC / UICC TNM staging guidelines, this staging method does not differ from the 1997 TNM system.

Primary tumor (T):

TX = Primary tumor cannot be assessed

T0 = No evidence of primary tumor

Ta = Non-invasive papillary carcinoma

Tis = Carcinoma in situ: "flat tumor"

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= Microscopically

T3b= Macroscopically (extravesical mass)

T4= Tumor invades any of the following: prostate, uterus, vagina, pelvic wall, abdominal wall

T4a= Tumor invades prostate, uterus or vagina

T4b= Tumor invades pelvic wall or abdominal wall

Regional lymph nodes (N):

NX = Regional lymph nodes cannot be assessed.

N0 = No regional lymph node metastasis.

N1 = Metastasis in a single lymph node in the pelvis (hypogastric, External iliac).

N2= Metastasis in amultiple lymph nodes in the pelvis (hypogastric,External iliac)

N3 = Metastasis in common iliac lymph nodes.

Distant metastasis (M):

M0 = No distant metastasis

M1 = Distant metastasis

Stage Grouping

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

Accurate pathological staging of bladder cancer remains a critical task that allows for appropriate prognostic and therapeutic stratification of patients.

Prognosis of the urinary bladder:-

Data on the prognostic importance of genetic changes for progression of UB carcinomas are largely missing because of the rarity of progression in these patients. In theory, molecular changes that decrease genetic stability are expected to herald poor prognosis in these patients, because an acquisition of multiple additional molecular changes may be required to transform non-invasive low grade neoplasia to invasive cancer. In fact, Tp53 alterations, known to decrease genomic stability, have been suggested as a prognostic marker in pTa tumours . Molecular parameters that were suggested to herald a particularly high risk of progression include Tp53 accumulation , reduced thrombospondin expression , loss of p63 expression , loss of E-cadherin expression , abnormal expression of pRb , LOH at chromosome 16p13 , as well as alterations of chromosomes 3p, 4p, 5p, 5q, 6q, 10q, and 18q (DS et al, 2001; Goddard JC, 2002; Urist MJ et al, 2002; Yoon)

Genes And Tumors:

The Human Genome Project taught us recently that we have about 30,000 genes. Of these, it is estimated that about 200 are involved in producing tumors . This does not mean, of course, that Nature equipped us with a set of genes programmed to generate cancer: what we mean here is that these genes can be misled into producing cancer (Bronchud MH,2000)

From Genes to Tumor:

If we examine the normal tasks of these cancer-related genes , we find that they fall into three groups: (a) oncogenes, which code for proteins that favor cell proliferation; (b) suppressor genes, which code for proteins that suppress cell proliferation; and (c) repair genes, which code for enzymes in charge of correcting any damage in the DNA. All the cells in the body depend on the careful work of these repair genes, which are especially important for cells that are constantly exposed to genotoxic agents, such as the keratinocytes exposed to ultraviolet light. The

oncogenes and suppressor genes are directly in charge of cell proliferation; they supervise checkpoints at given phases of the cell cyle, and control cell numbers by balancing cell birth and cell death (apoptosis). It has become customary to explain the malfunctions of these two sets of genes with an automobile metaphor. The oncogenes correspond to the accelerator: if it is jammed, the car will speed out of control. The suppressor genes correspond to the brakes: if they cease to function the car will again speed out of control. Both oncogenes and suppressor genes can be visualized as keepers of the cell cycle which explains why they are known collectively as gatekeeper genes Correspondingly, cancer can be considered as a disease of the cell cycle.(Vogelstein B& Kinzler KW, 1998)

At the level of cells, cancer is definitely inherited; cancer cells beget cancer cells, with rare exceptions. At the level of people, every type of cancer can be in the genes: what is inherited is not the cancer itself, but the predisposition to develop a given type of tumor. However, this familial predisposition is uncommon; it is involved in no more than a fraction of cancers: 0.1 to 10 percent, depending on the site. An optimist might add that even in families with dominantly inherited cancer, not all the gene carriers develop it, and 50 percent of family members do not carry the defective gene at all (Ponder BAJ, 1990).

For most of the common cancers there are cancer families in which cancer of one particular organ occurs with a higher incidence and at a younger age . This reminds us of the strains of commercially available mice that are guaranteed to develop a given percentage of tumors of a given organ at a given age. These strains tell us that cancer genes are there; in mice they are revealed by inbreeding.(King M-C, 1990).

Genetic Alterations:

Bladder cancer is a disease that results from molecular alterations, many of which have been defined. The earliest cytogenetic studies in bladder cancer showed evidence of alterations in chromosomes 9q, 11p, and 17p, the most common defects being allelic losses, loss of heterozygosity, and microsatellite alterations. In general, amplification of regions within chromosomes reflects the identification of possible oncogene loci, and allelic losses and loss of

heterozygosity reflect the possible presence of tumor suppressor genes (Gonzalez & Zulueta M, 1993).

Chromosome 17p, which harbors the Tp53 tumor suppressor gene, shows allelic losses in bladder cancer. The other tumor suppressor gene found altered in many bladder cancers is the retinoblastoma susceptibility gene Rb, located on chromosome 13q. Molecular evidence suggests the presence of two distinct pathways in urothelial tumorigenesis. Low-grade noninvasive superficial papillary tumors have alterations in chromosome 9q1 and exhibit activating mutations in the HRAS and FGFR3 genes, whereas flat carcinoma in situ (CIS) and invasive tumors have structural and functional defects in the Tp53 and Rb tumor suppressor pathways.. Tp53, Rb, and p16 are all involved in cell cycle control, and it has become clear that cell cycle regulators are crucial in bladder cancer progression (van Rhijn BW et al, 2002; Mitra AP et al, 2206).

On the basis of consistent and frequent genetic defects in bladder tumors and increasing understanding of cell cycle regulation and its role in tumor behavior, studies from many groups led to the description of a detailed and sophisticated model of important molecular events in bladder cancer (figure 2-26) (Orlow I et al, 1994; Mitra AP et al, 2006).

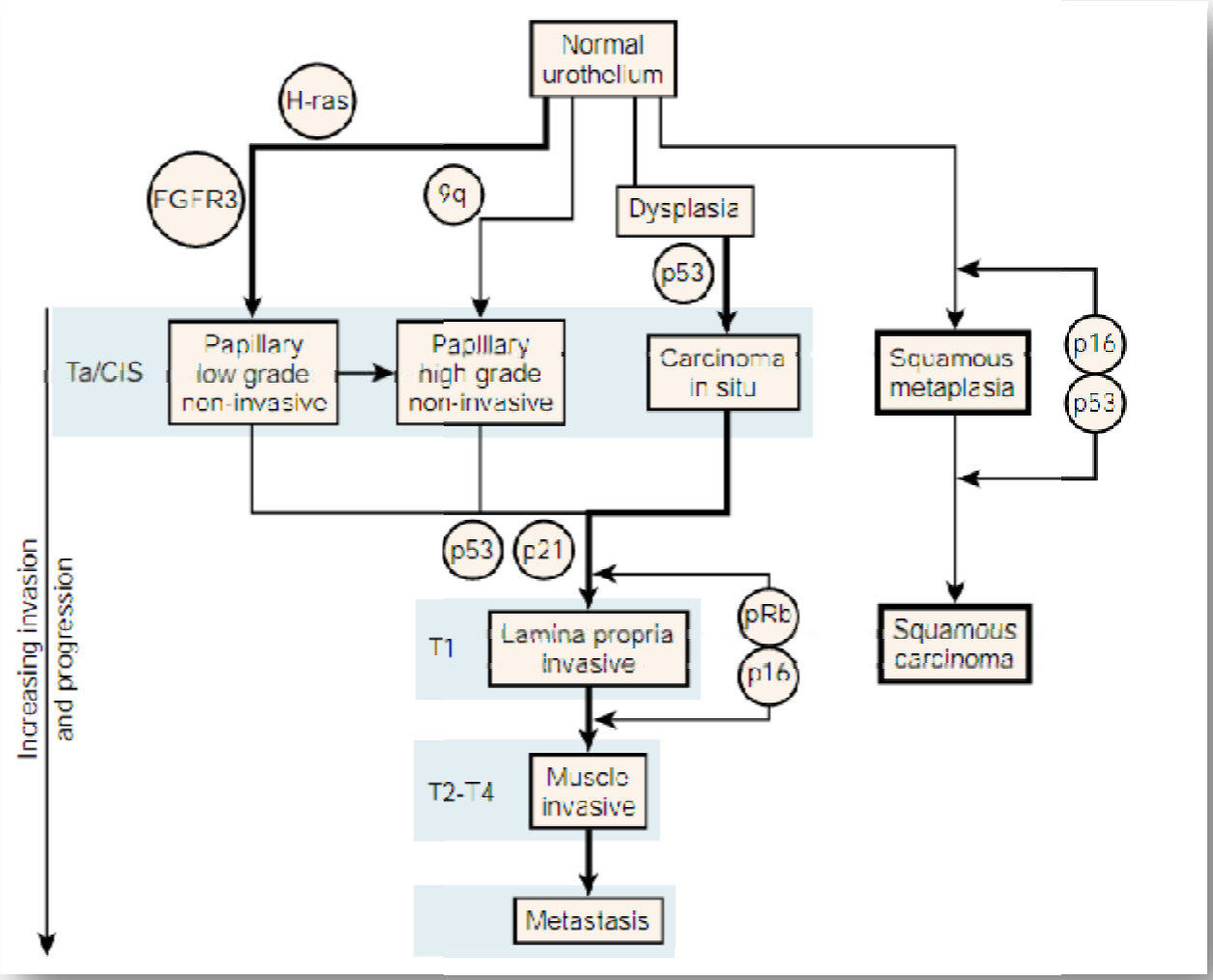


Figure (2-26): molecular pathways in invasive bladder cancer**,** Proposed model for bladder cancer tumorigenesis and progression. This model indicates some key molecular events that have been described and shows that papillary carcinoma, carcinoma in situ, and squamous metaplasia have unique molecular profiles. The location of the particular events illustrates genetic abnormalities that pose a risk for progression of a particular phenotype, rather than indicating the specific timing of that genetic alterationMost papillary noninvasive transitional cell carcinomas do not progressto an invasive phenotype.. **(J ClinOncol,2006).**

Cancer phenotypes:

Because there are so many kinds of tumors, many cancer-prone phenotypes can be expected. A fair complexion can be considered as a phenotype that predisposes to melanoma. Less obvious phenotypes concern the individual's enzymatic makeup: there can be inborn differences in the enzymes that activate or detoxify carcinogens, as well as differences in rates of DNA repair. For example, the poor acetylator phenotype predisposes dye-stuff workers to bladder cancer . Another example: certain individuals respond to polycyclic hydrocarbons in smoke by producing an inducible form of cytochrome P450 called P450 IA. This isoenzyme transforms the hydrocarbons into oxygenated intermediates that bind to DNA. Individuals with this trait appear to be prone to adenocarcinoma of the lung (Anttila S et al, 1991) .

These and many other findings have opened the new field of ecogenetics, which studies the interaction of genes with the environment (not to be confused with epigenetics, the study of gene-regulating activities that do not involve changes in the DNA code, e.g., gene regulation by chromatin . In summary, there is strong evidence for a genetic component in many forms of cancer, and even for cancer of the lung, which is typically environmental; but the mode of inheritance is little understood. Puttilo and co-workers have listed over 240 genetic conditions that predispose to cancer . We now turn to those rare forms of cancer that are definitely based on heredity, this will give us an opportunity to see how the cancer genes work (Bale AE & Brown SJ, 2001; Pennisi E, 2001).

Defects of the p53 gene:-

The Tp53 gene is a tumor suppressor gene that maps to the human chromosome 17p13.The product of the gene is a cellular phosphoprotein that has been shown to have tumor suppressive properties. Compared with the wild type protein, mutant Tp53 protein is more stable with a prolonged half-life and more likely to be detected by immunohistochemical analysis. Esrig et al demonstrated a strong association between the immunohistochemical detection of Tp53 nuclear accumulation in formalin-fixed, paraffin-embedded tissue and the presence of a mutation in the Tp53 gene as demonstrated by the method of single-strand conformational polymorphism.

Regardless of the mechanism for nuclear reactivity, accumulation of the protein is indicative of a change in the cell state, and detection of this change by immunohistochemistry has been shown to aid the diagnosis of malignant disease (Vogelstein, B. and K, W. Kinzler, 1992; Esrig, D et al, 1993).

Mutations involving the Tp53 gene have been found in a wide variety of malignancies including urothelial carcinomas. Mutations of the Tp53 gene and immunohistochemical positivity for the p53 protein have been found in 40% to 60% of urothelial carcinomas. Many investigators have demonstrated a positive correlation between tumor expression of Tp53 and pathologic indicators of progression in urothelial carcinoma, including high grade and stage Fujimoto reported that the incidence of Tp53 mutations is much higher in invasive and high-grade urinary bladder cancers than in superficial and low-grade tumors. Likewise, Tp53 mutations to be associated with higher grades and later stages of bladder tumors. Although these studies have suggested that mutations of the Tp53 gene are involved in late events of tumorigenesis, other studies have demonstrated changes in Tp53 expression in premalignant noninvasive lesions, including those of the oral cavity, bronchial mucosa, and esophagus .Some studies demonstrated a strong association of Tp53 staining between dysplasias and urothelial carcinomas, indicating that similar Tp53 mutations may be seen in the preinvasive stage of bladder neoplasia (Fujimoto, K et al, 1992; Soini, Y et al, 1993; Harano, H et al, 1999)

Chapter 3

Patients & METHODS

Patients:

From 2007 to 2011, 50 Libyan patients (40 males, 10 females) with urinary bladder carcinoma were retrospectively studied. Age ranging from 40 to 81 years (mean 63.95 years).The carcinomas were retrieved from the Department of Pathology, Faculty of Medicine, Benghazi University's archival files based on available paraffin blocks. One pathologist reviewed all the tumors H&E stained slides. For each tumor, histopathological criteria from the world health organization classification 2004 were used and staging was made according to the American joint committee on cancer system. All samples were stained by H&E and immunohistochemistry. Tonsils tissue used as an external positive control Table (3-1)

Immunohistochemical method

Paraffin embedded blocks of primary urinary bladder carcinoma were obtained from pathology department archive. Sections were cut serially at 5µm for immunohistochemical (IHC) Staining .

Table3-1.Clinicopathological characteristics of the patients

Characteristic	No .of patients	Percentage (%)
Gander		
Male	**40**	**80**
Female	**10**	**20**
Age(yrs)		
Range	**(40-81)**	
mean	**63.98**	
Meadian	**66.5**	
Histopathological grade		
High	**19**	**38**
Low	**31**	**62**
Type		
Tcc*	**47**	**94**
Ac*	**1**	**2**
Scc*	**2**	**4**
Primary tumor status		
T1	**35**	**70**
T2	**11**	**22**
T3	**1**	**2**
Tx	**3**	**6**

* Tcc is transitional cell carcinoma.

*Ac is adnocarcinoma.

*Scc is squamous cell carcinoma.

Immunohistochemical method

Immunohistochemistry (IHC) is microscopic localization of specific antigens (eg, proteins) in tissues by staining with antibodies labeled with fluorescent or pigmented material. Immunohistochemistry is used to determine the origin of a tumor as well as the prognosis and treatment. It is a critical technique used by the pathologist to assist in making a final diagnosis.

Paraffin embedded blocks of primary urinary bladder carcinoma were obtained from pathology department archive. Sections were cut serially at 5µm for immunohistochemical (IHC) Staining .

Immunohistochemical staining:

Kits used in LIF Immunostaining

I- Primary antibody:

- **Source:** Zymid's monoclonal Mouse **anti p53 (clone: Bp53,12)**, 6.0 ml predilute antibody, ready to use. Zymid Laboratories Invitrogen Immunodetection.
- **Product:** Mouse anti-p53 is purified from mouse ascites and diluted in phosphate buffered saline(PBS), pH 7.4 and 1% bovine serum albumin(BSA) with 0.1% sodium azide (NaN3) as a preservative.

II- Specificity: Recombinant human wild-type p53 protein, total protein concentration:10gm/L, isotype: IgG Kappa, antibody concentration: 2.4mg/L. It reacts with both mutant and wild-type forms of p53. It was raised against recombinant human

wild-type p53 protein. It is suitable for immunostaining of formalin- fixed, paraffin-embedded tissue.

III-Universal kit:

- Intended use: Universal DAKO LSAB® kit, peroxidase (DAKO LSAB®+- HRP). This kit offers an enhanced signal generating system for the demonstration of antigens in paraffin-embedded tissue, cryostat tissue and cell preparations. This is by sequential application of bio-tinylated link antibodies to primary antibodies followed by a complex of streptavidin and bio-tinylated peroxidase. Finally, the antigen antibody complex is visualized using the provided substrate chromagen solution.

III- Reagents: This kit consists of labelled streptavidin biotin (LSAB) reagents which include:

1- 15 ml of 3% Hydrogen peroxide: 3% hydrogen peroxide in water.

2- 15 ml of Link: Bio-tinylated anti-rabbit, anti-mouse and anti-goat immunoglobulins in phosphate-buffered saline (PBS) containing carrier protein and 0.1 % sodium azide.

3- 15 ml of Streptavidin Peroxidase: Strepatavidin conjugated to horseradish peroxidase in PBS containing carrier protein and 0.01% thimerosal.

4- 18 ml of Buffered Substrate: Buffered substrate solution, pH 7.5, containing hydrogen peroxide and a preservative.

5- 1 ml of Dab Chromogen: 3.3- diaminobenzidine chromogen solution, resulting in a brown-coloured precipitate at the antigen site.

IV- Other reagents:

1- Xylene and ethanol.

2- Distilled water.

3- Counter stain: Light Mayer's Haematoxylin.

4- 37mM ammonium hydroxide.

5- Mounting media: DAKO® Faramount Aqueous Mounting Media (Code No. S 3025).

Immnostaining procedure:

Pretreatment:

1- **Deparaffinize slides**
2- Wash slides with distilled water 3 times for 2 min each.
3- Place a glass beaker containing 500ml of citrate buffer (Cat. No. 00-5000) on a hot plate. Heat the buffer solution until it boils.
4- Put the slides in a slide rack and place in the beaker with boiling buffer. Keep it boiling for 15 minutes.
5- After heating, remove beaker from the hot plate and allow it to cool down for at least 15-20 minutes at room temperature
6- Rinse slides with PBS (Cat. No. 00-3000) and begin immunostaining protocols

Staining protocol:

Step 1: 3% Hydrogen Peroxide:

- Tap off excess water and carefully wipe around specimen. Apply enough drops from Bottle 1 (3% hydrogen peroxide) to cover specimen.
- Incubate 5 minutes.
- Rinse gently with wash solution from a wash bottle and place in fresh buffer bath.

Step 2: Primary Antibody and Negative Control Reagent:

- Tap off excess buffer and wipe slide as before.
- Apply enough prepared primary antibody or negative control reagent to cover specimen.
- Incubate 60 minutes.

- Rinse slide as in step 1.

Step 3: Link:

- Immediately tap off excess buffer and wipe slide as before.
- Apply enough yellow drops from bottle 3 (link) to cover specimen.
- Incubate 15 minutes.
- Rinse slide as before.

Step 4: Streptavidin Peroxidase:

- Wipe slide as before.
- Apply enough RED drops from bottle 4 (streptavidin) to cover specimen.
- Incubate 15 minutes.
- Rinse slide as before.

Step 5: Substrate-Chromogen Solution:

- Wipe slide as before.
- Apply enough of the prepared substrate-chromogen solution to cover specimen.
- Incubate 5 minutes.
- Rinse gently with distilled water from wash bottle.

Step 6: Counter-stain:

- Place slides in a bath of haematoxylin.
- Incubate for 2-5 minutes, depending on the strength of the haematoxylin used.
- Rinse gently with distilled water from a wash bottle.
- Dip 10 times into a wash bath filled with ammonia water.
- Place in distilled or de-ionized water for 2 minutes.

Step 7: Mounting:

- Specimens mounted and cover-slipped with an aqueous-based mounting medium (DAKO® Faramount Aqueous Mounting Media Code No. S 3025).

Interpretation of staining:

The positive control specimen (breast carcinoma, colon carcinoma) was examined for a brown-coloured nulei. The presence of this colour can be interpreted as a 3+ positive staining result indicating that the kit reagents are performing properly. In negative control specimen, there was absence of specimen staining. Examination for any non-specific staining present on the negative control reagent slide was done. Non-specific staining, if present, was of rather diffuse appearance.

The test specimens stained with the primary antibody were then examined. The presence of brown-coloured nuclei was interpreted as a positive staining result, while the absence of nuclear staining was interpreted as a negative staining result.

TP53expression pattern in low grade urothelium carcinoma,and high grade urothelium carcinoma are illustrated in figures (3-1to 3-7) , the immunohistochemical staining of Tp53 was not successful in all 50 cases.

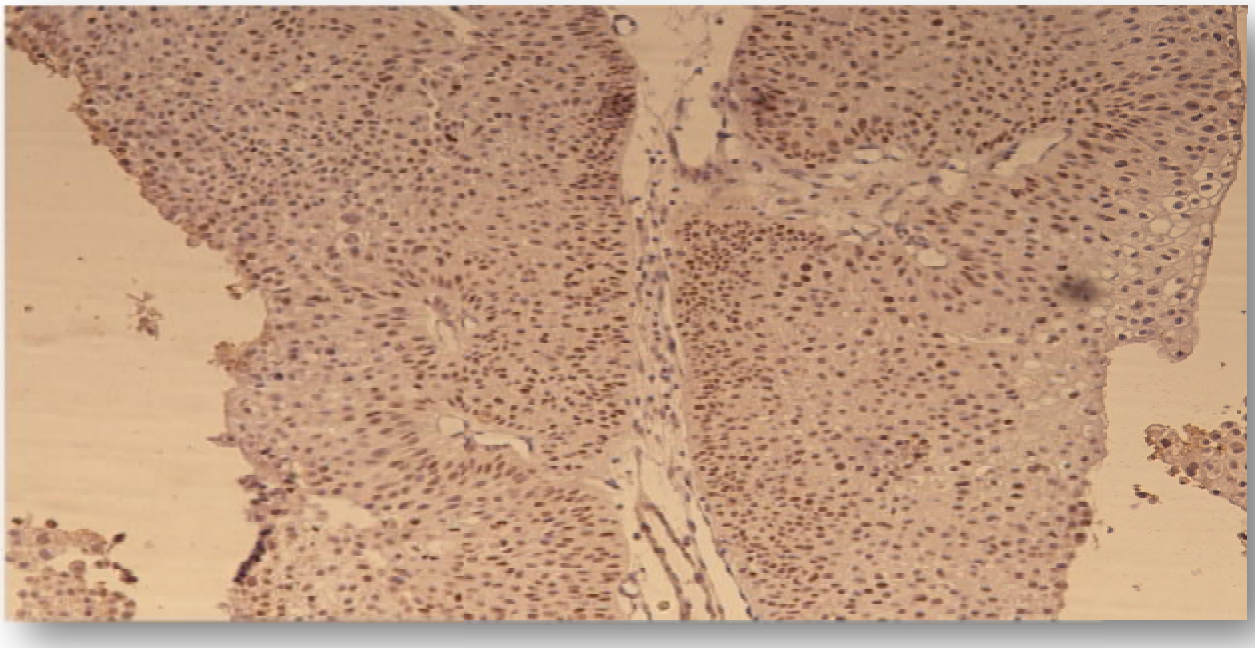


Figure (3-1): Superficial low grade Urothelial carcinoma Tp53 index 1.5(x4).

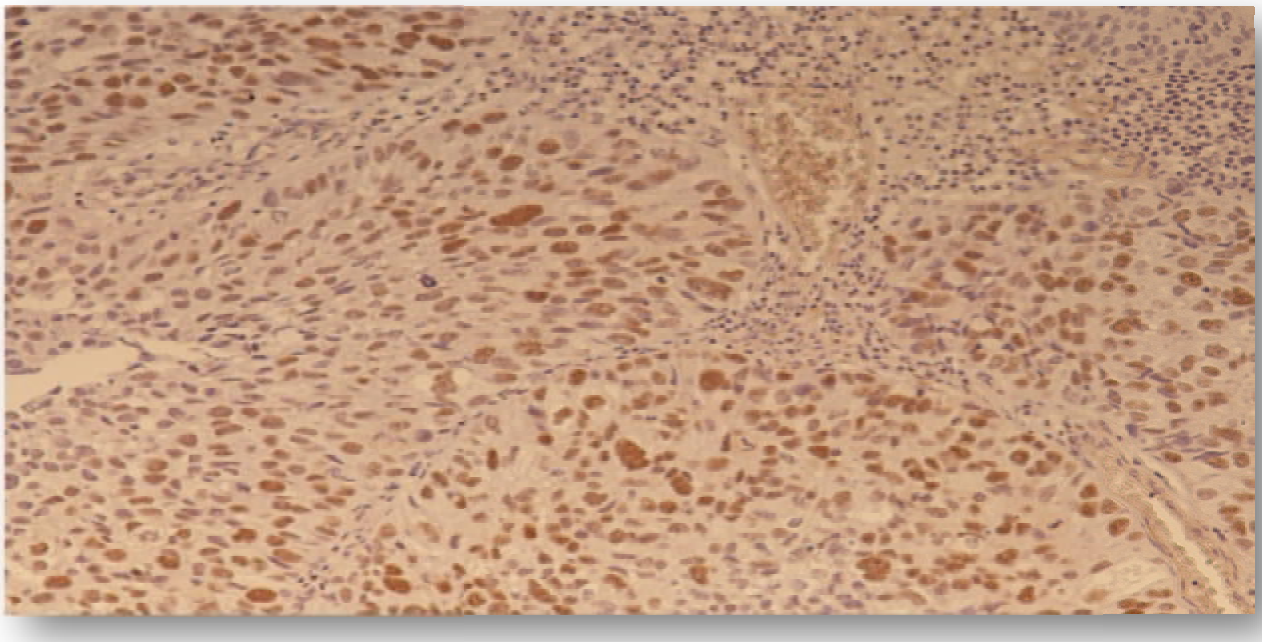


Figure (3-2): Superficial low grade Urothelial carcinoma Tp53 index 1.5(x10).

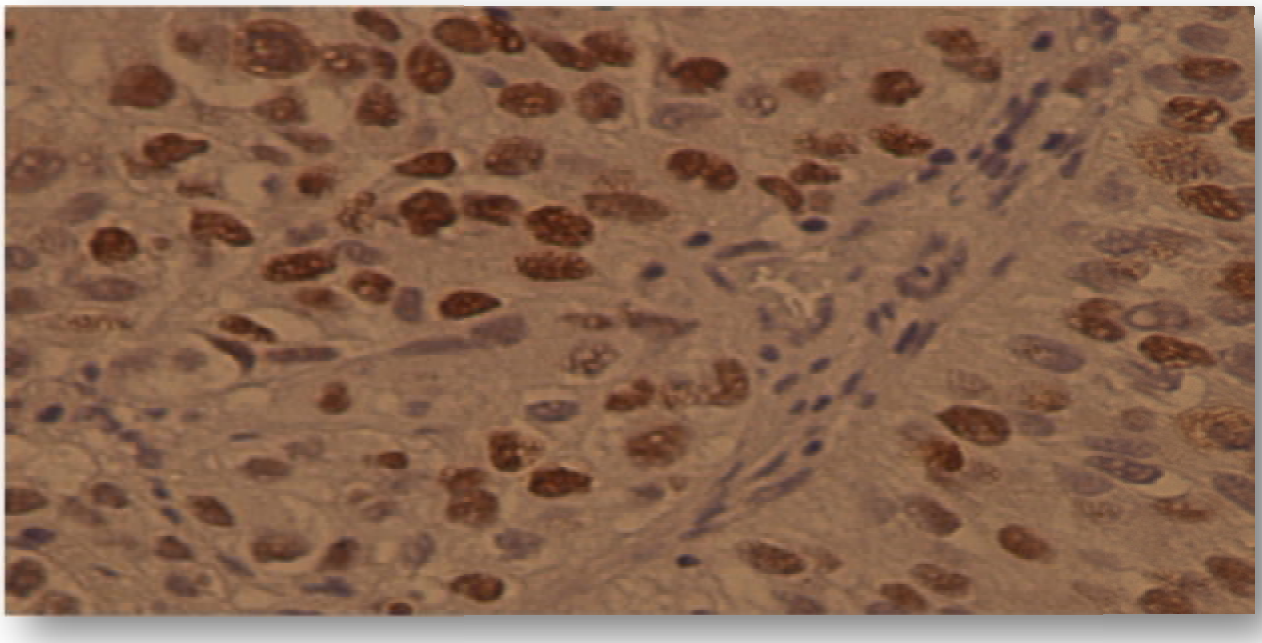


Figure (3-3): Superficial low grade Urothelial carcinoma Tp53 index 1.5(x40).

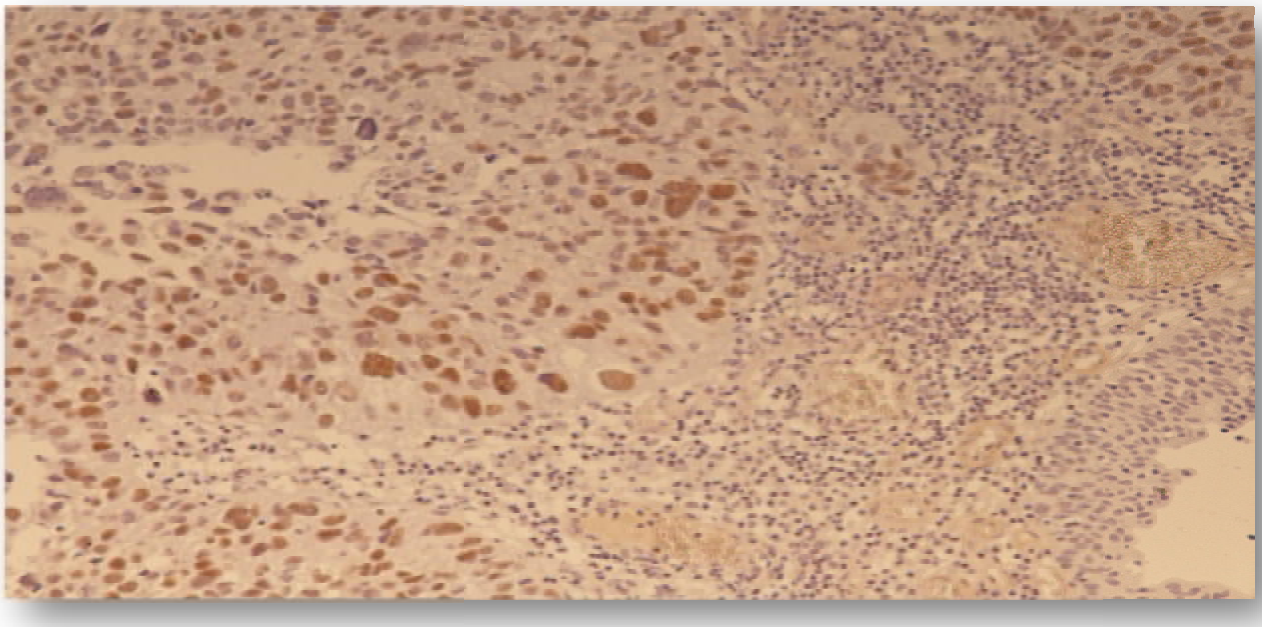


Figure (3-4): Invasive low grade urothelial carcinoma Tp53 index 1(x10).

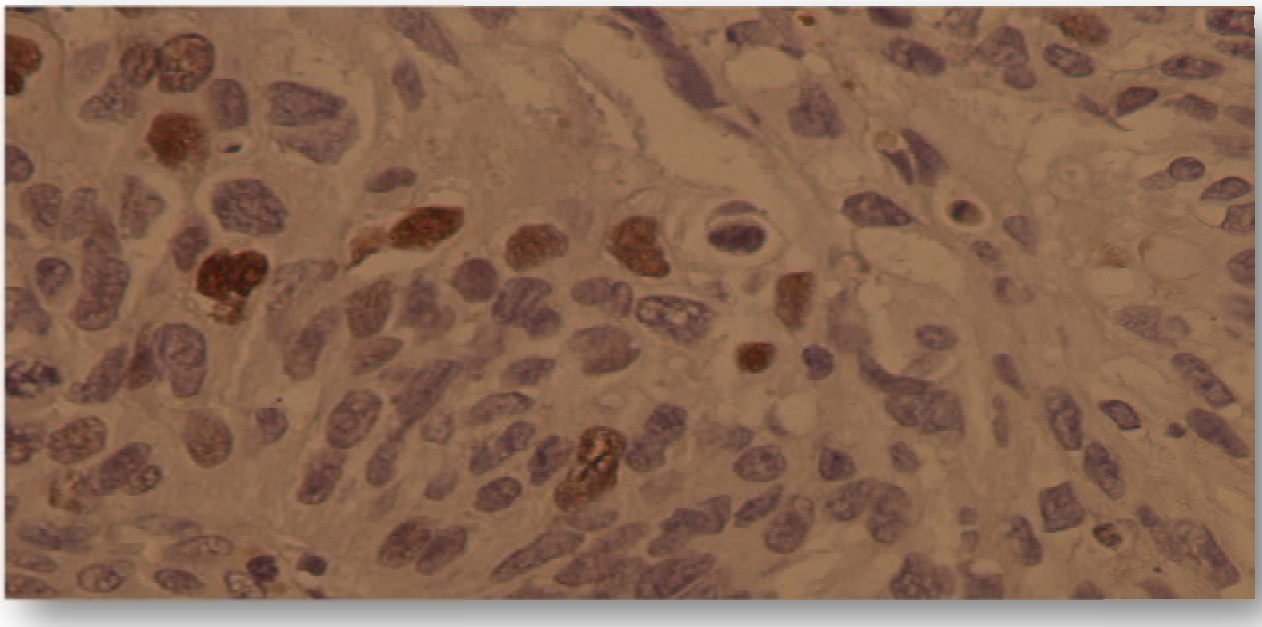
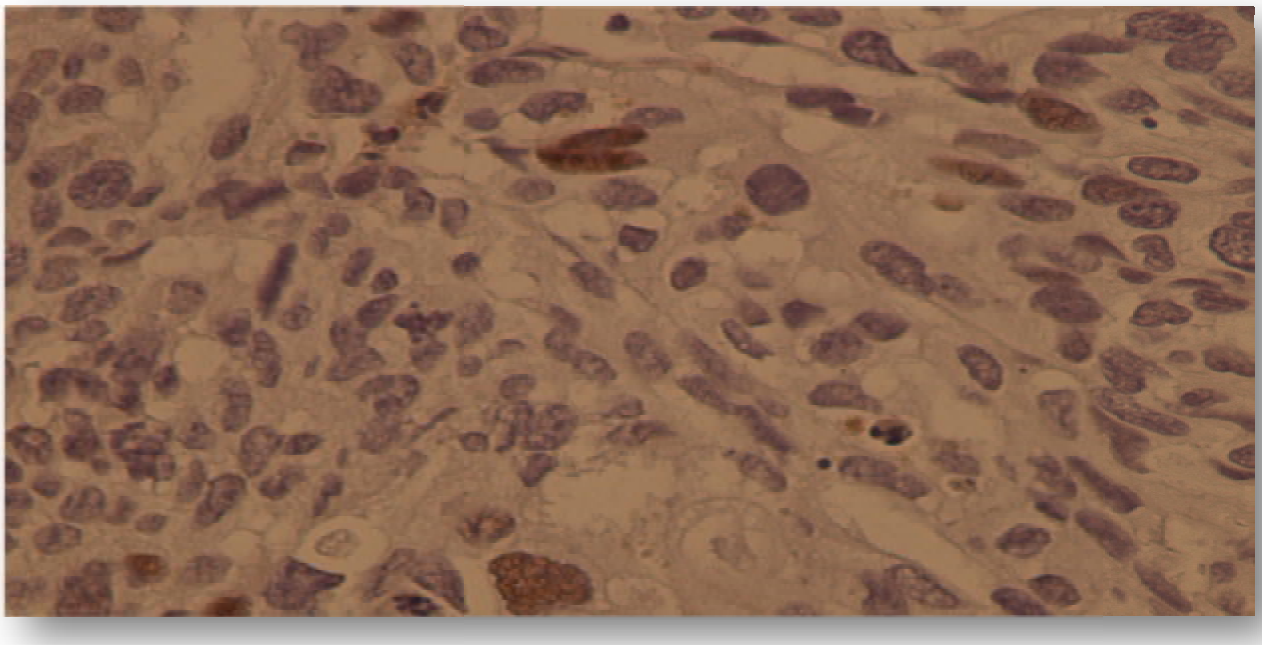


Figure (3-5): superficial high grade urothelial carcinoma Tp53 index 0.5 (x40).



Figure(3-6) : Invasive high grade urothelial carcinoma Tp53 index 1 (x40).

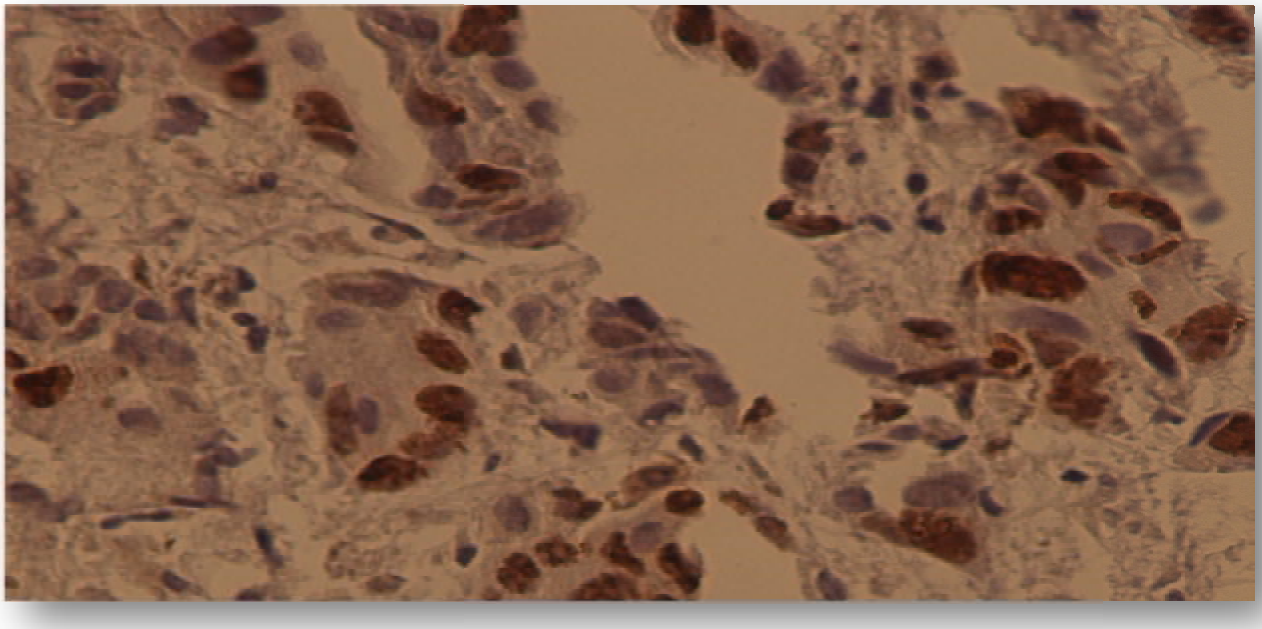


Figure (3-7) : Invasive high grade urothelial carcinoma Tp53 index 2 (X40).

Scoring system of p53:

P53 staining positivity was indicated by the presence of nuclear brown staining. Nuclear counting was performed in areas with maximal staining intensity within the tumors foci away from any areas of artifact, necrosis, or inflammation and without prior knowledge of patient's clinicopathologic outcome.

Nuclear expression patterns:

3+++ No blue to be seen through brown staining, nuclei appear darker than cytoplasm.

2++ Blue scarcely to be seen through brown staining , nuclei appear darker than cytoplasm.

1+ Blue clearly to be seen through brown staining.

0 only blue staining.

The most intensively stained area was chosen by low power light microscopy at x4 magnification , and the percentage of each intensely stained field was counted at x40 magnification power .

Score of each slide case was calculated by the following equation:

$$I = F0\ X0 + F1X1 + F2X2 + F3X3$$

Where **I** is the staining index, f0 – f3 are the fractions of the cells showing a defined level of staining intensity (from 0 to 3). Theoretically, the index could vary between 0 and 3.(Abdalla F,2008).

Statistical analysis:-

Statistical analyses were performed using the IBM SPSS® Statistics(IBM Company, New York , NY, USA) and STATA (StataCorp.,TX , USA) software packages (IBM PASW Statistics for Windows, version 19 and STATA /SE 11.1).Frequency tables were analyzed using the chi-squared test, with likelihood ratio or Fischer's exact test being used to assess the

significance of the correlation between the categorical variables.Odds ratio and their 95% confidence intervals (95% CI) were calculated where appropriate, using the exact method. Differences in the means of continuous variables were analyzed using non-parametric tests(Mann- Whitney or Kruskal- Wallis) for 2- and multiple independent samples, respectively. Analysis of variance (ANOVA) was only used for deriving the mean values (and their 95% CI) of each individual stratum.

p < 0.05 were regarded statistically significant.

Chapter 4

RESULT

&

DISCUSSION

Results

Patients clinicopathological data :-

1- Age :- It was found that 21 (42%) of patients are under 63.98years (mean) and 29 (58%) of patients above 63.98 years, (Figure 4-1).

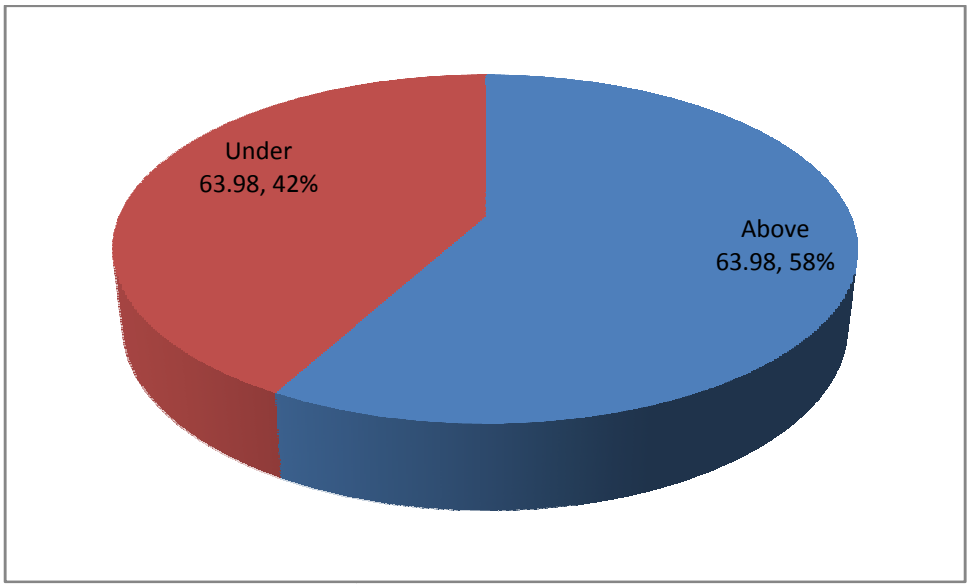


Figure (4-1)

2- Gender:- 40 (80%) of patients were males and 10 (20%) of patients were females, (Figure 4-2)

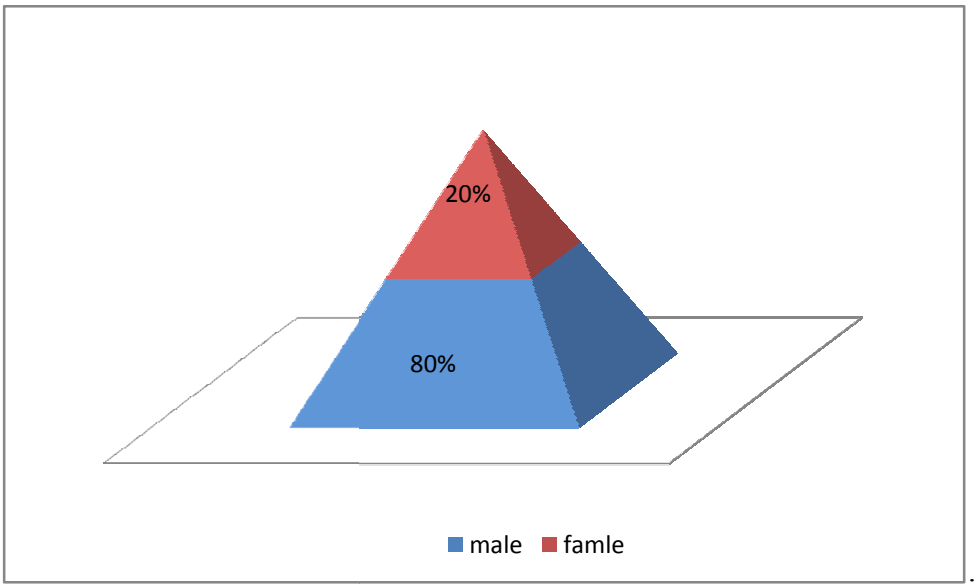


Figure (4-2)

3- The type :- 47 (94%) of cases was transitional cell carcinoma,and 1(2%) adenocarcinoma,and 2 (4%) squamous cell carcinoma, (Figure 4-3).

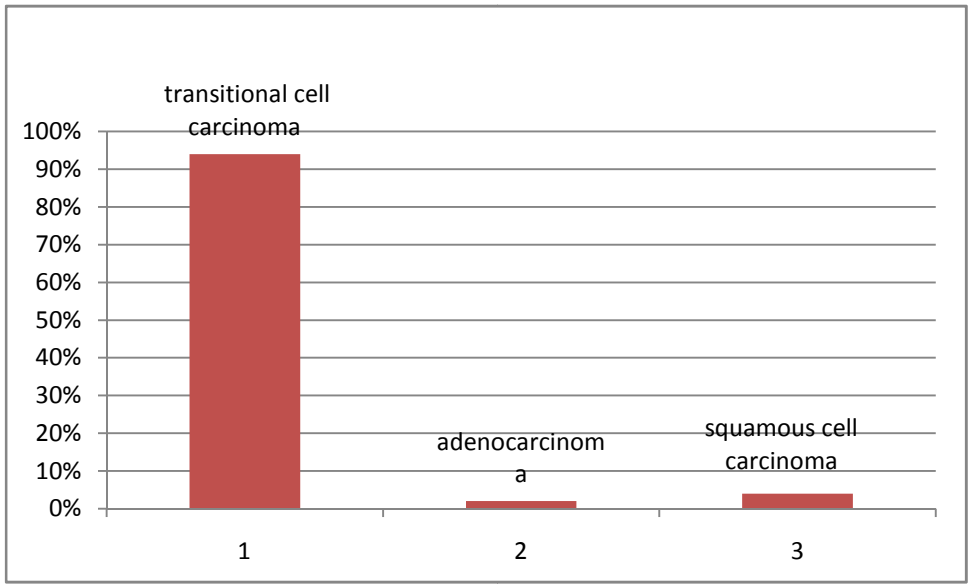
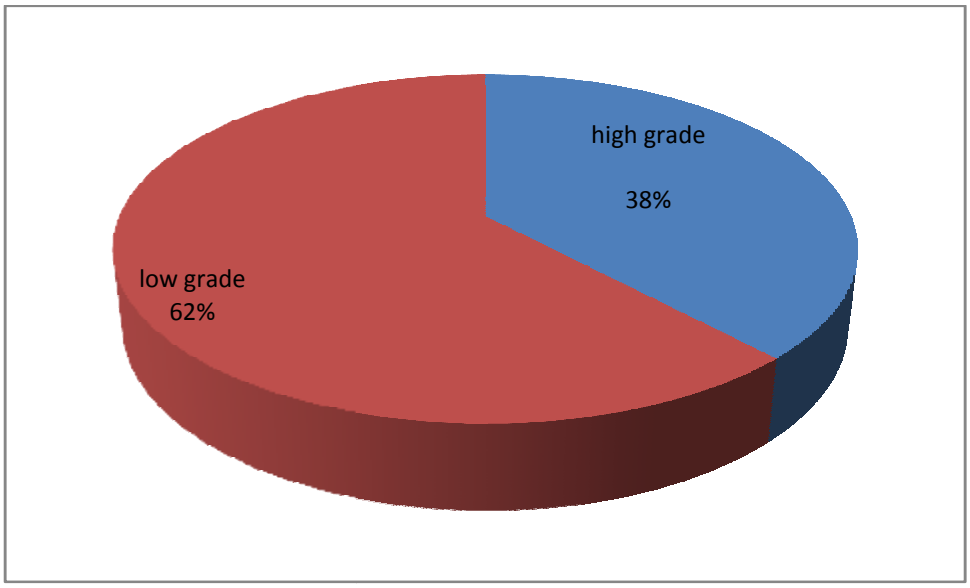


Figure (4-3)

4-Grade :-19 (38%) of cases was high grades, 31 (62%) of cases was low grades,(Figure4-4)



Figure(4-4)

5-Invasion:- 3 (6%) of cases was Ptx, and 35 (70%) of cases was Pt1, and11 (22%) of cases was Pt2 ,and1 (2%) of cases was Pt3 (Figure4-5).

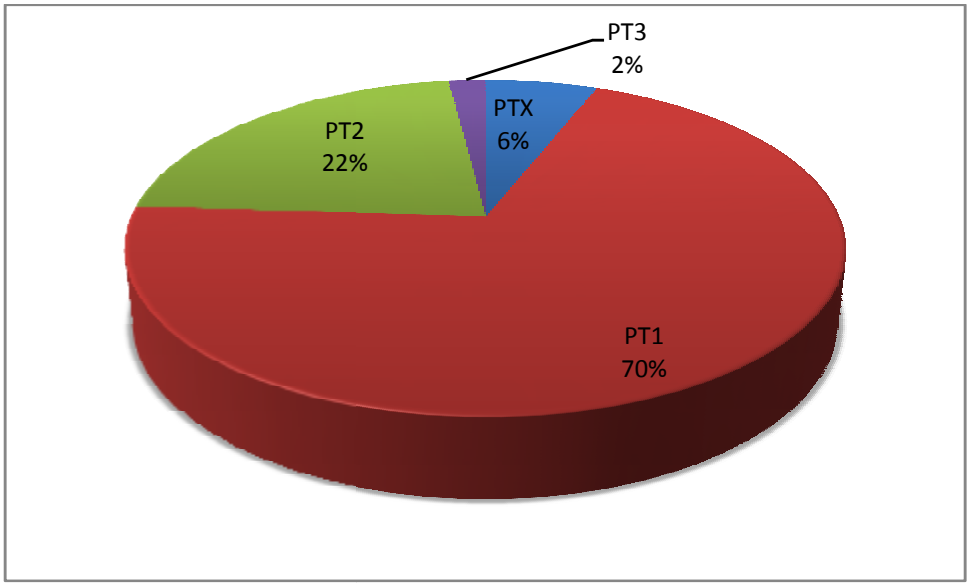


Figure (4-5)

TP53 expression related to clinicopathologic features:

Out of 50 urinary bladder carcinomas 10 cases (20%) p53 staining were considered negative (staining intensity= 0),whereas 40 cases (80%) were considered positive (staining intensity +1or +2). While score 3 has not been detected completely.

NOTE:

Score 1 =34 cases (68%).

Score 2 =6 cases(12%).

P53 expression was statistically analyzed in relation to all available clinical variables and tumor characteristics . The clinical variables recorded were age, sex, grading ,and staging. A significant correlation between Tp53 expression and age was observed (p=0.004 , chi-square, p=0.003, ANOVA). No other significant correlations between Tp53 expression and the other clinicopathological variables were observed, see tables (4-2,4-3).

Table (4-2) P-value of significance of Pearson chi-square test of TP53 expression in 3 systems with correlation to each clinicopathologic data .

Variables	P value 0,1,2,3	P value 0,1 vs 2,3	P value 0 vs 1,2,3
Age	0.015	0.004	0.567
Gender	0.464	0.571	1
Grade	0.398	0.963	0.197
T Invasion	0.456	0.655	0.304

Note: p value ≤ 0.05 is significant

p value ≥ 0.05 is not significant

Table(4-3) .Results of the correlation between clincopathological data and the mean of Tp53 expression by ANOVA .

Variables	Significance 0,1,2,3	Significance 0,1 vs 2,3	Significance 0 vs 1, 2,3
Age	0.012	0.003	0.576
Gender	0.485	0.580	1
Grade	0.417	0.964	0.205
T Invasion	0.415	0.368	0.422

-Immunohistochemical examination methods were employed to investigate Tp53 expression in 50 TURBT specimens of UB carcinoma.

-Interestingly, Tp53 expression resulting in significant observation with age (P=0.004 chi square , 0.003 anova). This result has yielded a conclusion of that a more advanced age group of patients is positively correlated with the Tp53 stains.

-While the UB carcinoma Tp53 stain showed an insignificant results with gender (P=0.464 chi square, 0.485 anova). This result makes a deduction that there was no relation between gender and p53 status.

- No correlation was found between Tp53 scoring and histological grade of tumor differentiation (P=0.197 chi square, 0.205 =anova).

-UB carcinoma resulting in no significant with invasion was (P=0.304 chi square, 0.368 anova), This result makes a deduction that there was no relation between invasion and Tp53 stains.

-H&E stain slides:

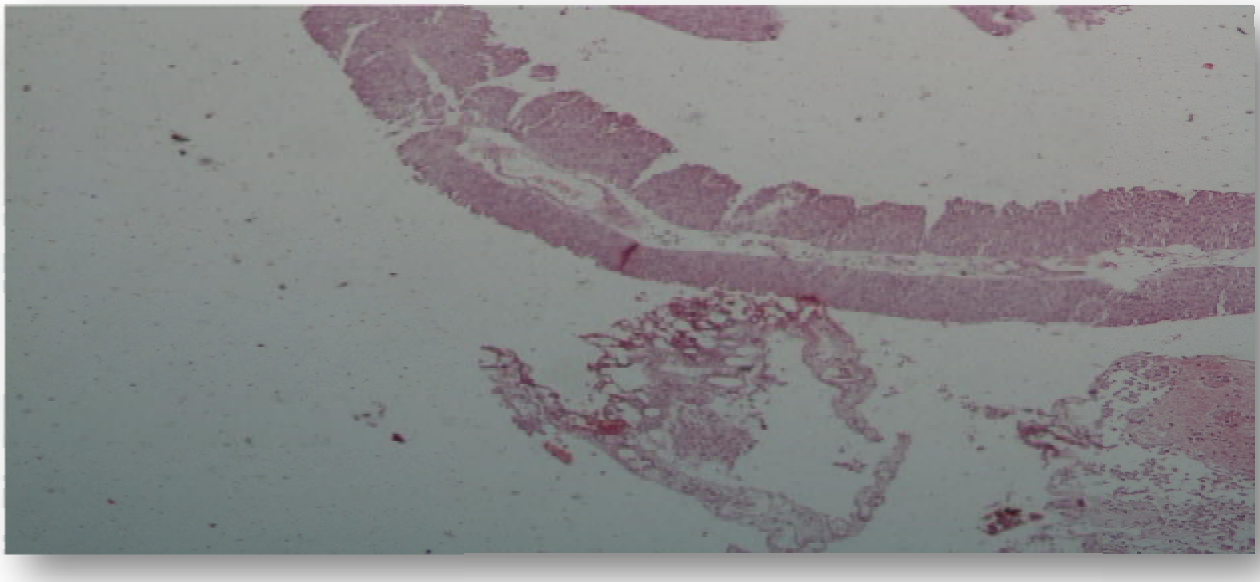


Figure (4-6): Superficial low grade Urothelial carcinoma, H&E stain (x4).

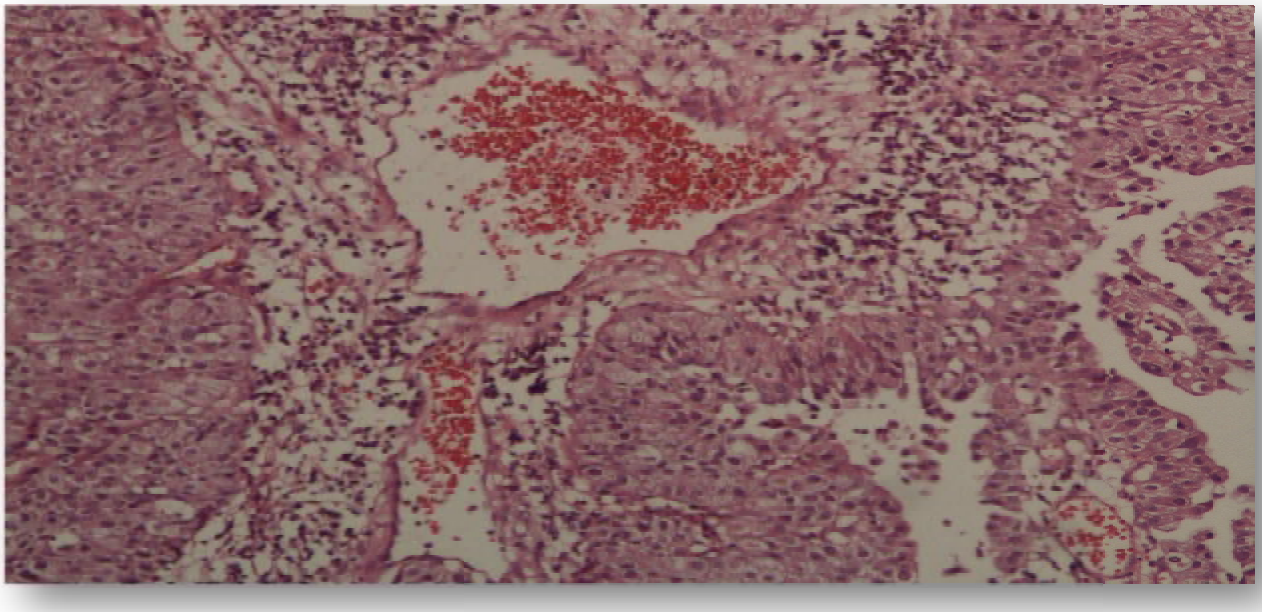


Figure (4-7): Superficial low grade Urothelial carcinoma, H&E stain (x40).

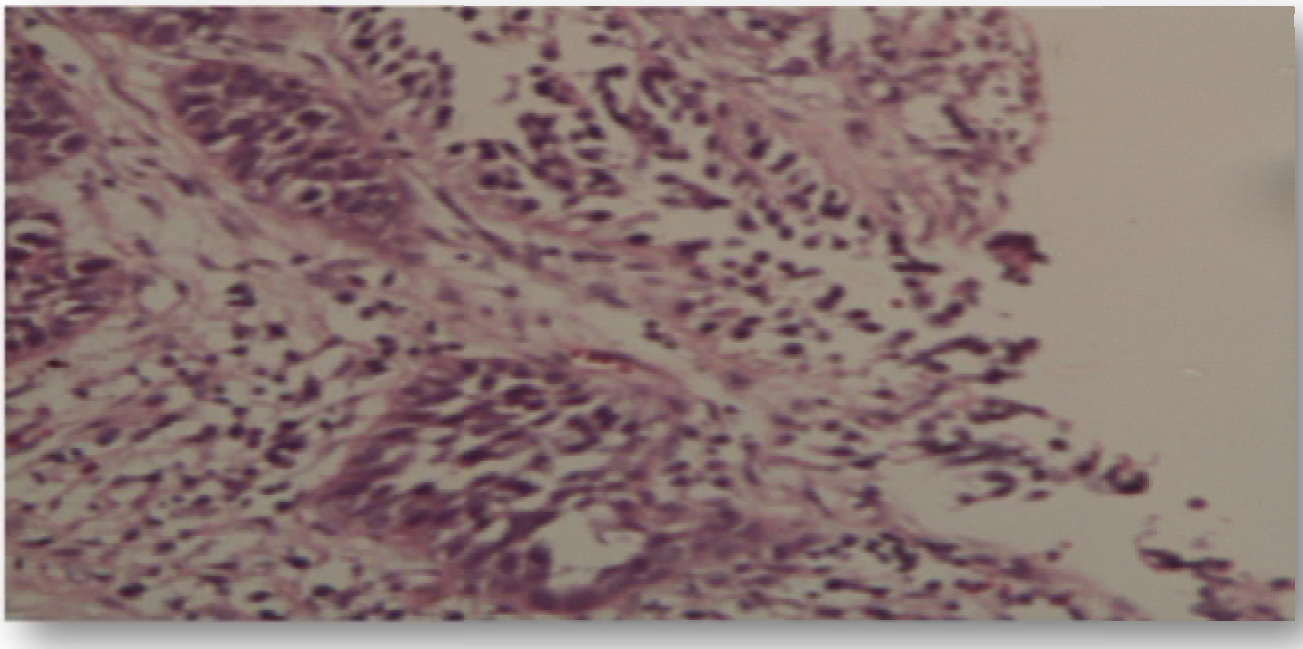


Figure (4-8): Invasive low grade Urothelial carcinoma, H&E stain (x40)**.**

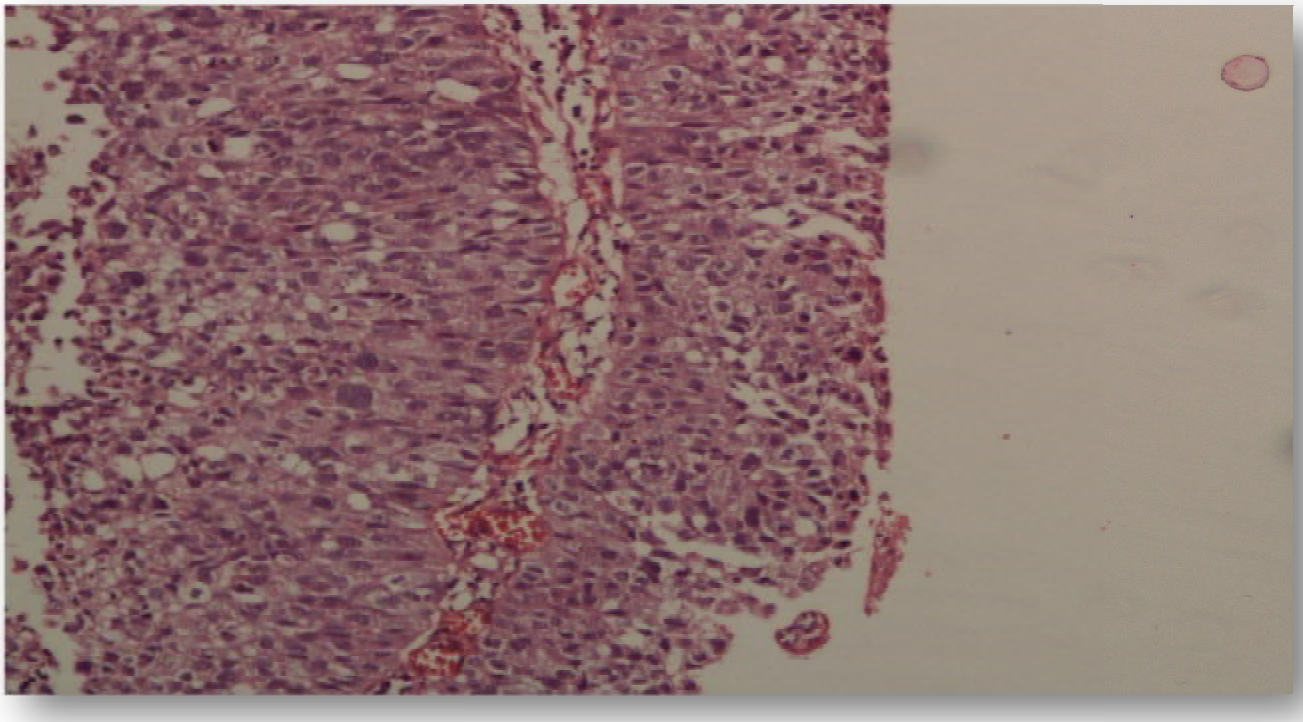


Figure (4- 9): Superficial high grade Urothelial carcinoma, H&E stain (x10).

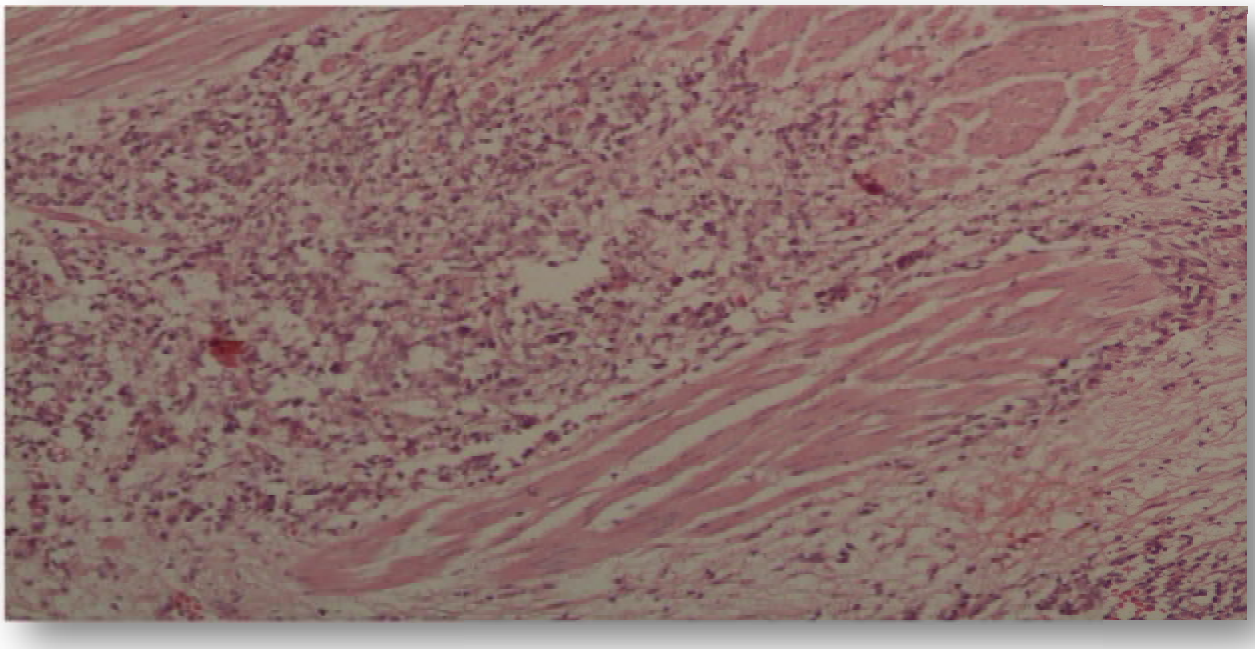


Figure (4-10) : Invasive high grade Urothelial carcinoma, H&E stain (x10).

DISCUSSION:

Although, Urinary bladder cancer is the most common form of urological malignancy in certain parts of the world, relatively little is known about the molecular occurrences leading to its development. TP53 is widely accepted as the most important prognostic factor in urinary bladder cancer to date.

Some data have shown that allelic loss of chromosome 17p and mutations of the Tp53 gene are common in certain human tumours, including lung and colon tumors . In contrast, the significance of Tp53 expression in UB carcinomas remains a point of controversy and few initial studies have described a correlation between increased Tp53 expression and reduced survival rates. (Starzynska and Bromery, 1992).

Therefore in this study, immunohistochemical examination methods were employed to investigate Tp53 expression in 50 TURBT specimens of UB carcinoma Tp53 expression resulting in significant observation with age (P=0.004 chi square , 0.003 anova). This result has yielded a conclusion of that a more advanced age group of patients is positively correlated with the Tp53 stains.

While the UB carcinoma Tp53 stain showed an insignificant results with gender (P=0.464 chi square ,0.485 anova). This result makes a deduction that there was no relation between gender and Tp53 status, a clue was agreed with those of (Vardar E et al , 2006) study. However, this is disagreed with (Lipponen P et al, 1994) results .

As (N Ibrahimet al ,2009),(Sunanda J al ,2004),and(david esring et al ,1994) no correlation was found between Tp53 scoring and histological grade of tumor differentiation (P=0.197 chi square ,0.205 =anova) . (Abd El-Maqsoud NM, Tawfiek ER ,2010) showed that tumours with p53 positive staining had a higher proliferative activity than did that stained negative.

UB carcinoma resulting in unsignificant with invasion was(P=0.304 chi square ,0.368 anova), This result makes a deduction that there was no relation between invasion and Tp53 stains, This

observation was confirmed in studies by (N Ibrahimet al ,2009), unfortunately are disagree with (Sunanda J et al ,2004),and(david esring et al ,1994).

Summary

* The urinary bladder is the most common site of urinary tract tumors, Tumors are more common in men than in women.

* In general, individual prognosis of infiltrating bladder tumors can be poorly predicted based on clinical factors alone. Tumor multifocality, tumour size of >3 cm, and concurrent carcinoma in situ have been identified as risk factors for recurrence and progression

* Approximately 90% of urinary bladder tumors in the United States are transitional cell type, 7% are squamous, 2% are glandular, and 1% are undifferentiated.This distrbution is in contrast to Schistosoma haematobium–endemic areas, such as Egypt and parts of the Middle East, where squamous cell carcinoma is the most common form of bladder cancer.

* In libya, bladder cancer prevalence accounting for about 8% of the total cancer cases and was ranked the 3rd after breast cancer(10.6%) &colorectal cancer(9.1%) in study on the incidence of cancer in eastern part of Libya conducted in the period between 1991&1995. About 327 cases of genitourinary tract cancer were diagnosed in males out of which 149were in the urinary bladder (45.6%) ,meanwhile 302 cases of genitourinary tract cancers were diagnosed in females only 25 cases were in the urinary bladder (8.3%).

*The TP53 gene is a tumor suppressor gene that maps to the human chromosome 17p13.The product of the gene is a cellular phosphoprotein that has been shown to have tumor suppressive properties. Compared with the wild type protein, mutant Tp53 protein is more stable with a prolonged half-life and more likely to be detected by immunohistochemical analysis**.**

*TP53 Alterations of the TP53 tumor suppressor gene have been by far the most intensively studied potential prognostic marker . Early studies suggested a strong prognostic importance of immunohistochemically detectable nuclear TP53 protein accumulation in both pT1 and pT2-4 cancers .

*Mutations involving the Tp53 gene have been found in a wide variety of malignancies including urothelial carcinomas. Mutations of the Tp53 gene and immunohistochemical positivity for the Tp53 protein have been found in 40% to 60% of urothelial carcinomas. Many investigators have demonstrated a positive correlation between tumor expression of Tp53 and pathologic indicators of progression in urothelial carcinoma, including high grade and stage.

* in this study, immunohistochemical examination methods were employed to investigate Tp53 expression in 50 TURBT specimens of UB carcinoma Tp53 expression resulting in significant observation with age only (P=0.004 chi square , 0.003 anova). This result has yielded a conclusion of that a more advanced age group of patients is positively correlated with the Tp53 stains.

Recommendation

The majority of published studies have shown a positive correlation between clinicopathologic data of urinary bladder carcinoma and Tp53 stain . A minority of studies had not demonstrated any association between urinary bladder carcinoma and Tp53 stain and this may be attributed to significant differences in the methodologies employed for:

1.Sample selection,

2. Immunostaining techniques,

3.Nuclear counting, (Evaluation & Expression)

4. Follow up data,

5.Small sample number ,

6. A number of biological and molecular differences may account for the discrepancy.

The reference

1. Abdalla F, Boder J , Buhmeida A, Hashmi H , Elzagheid A , Collan Y.Nuclear morphometry in FNABs of breast disease in Libyans. Anticancer Res 2008; 28:3985-9.

2. Abrahams NA, Moran C, Reyes AO, et al: Small cell carcinoma of the bladder: A contemporary clinicopathological study of 51 cases. Histopathology 2005; 46:57-63, .

3. Al- fituri Omran Incidence of cancer in the eastern part of Libya, 4th Libyan surgeon society conference, musrata- Libya, 1995.

4. Alsheikh A, Mohamedali Z, Jones E, Masterson J, Gilks CB. Comparison of the WHO/ISUP classification and cytokeratin 20 expression in predicting the behavior of low-grade papillary urothelial tumors. Mod Pathol 2001; 14: 267-272.

5. Amin MB, Gomez JA, Young RH: Urothelial transitional cell carcinoma with endophytic growth patterns: A discussion of patterns of invasion and problems associated with assessment of invasion in 18 cases. Am J Surg Pathol 1997; 21:1057-1068,.

6. Amin MB, Grignon DJ, Humphrey PA, Srigley JR. Gleason Grading of Prostate Cancer: A Contemporary Approach. Philadelphia: Lippincott Williams & Wilkins; 2004.

7. Amin MB, McKenney JK: An approach to the diagnosis of flat intraepithelial lesions of the urinary bladder using the World Health Organization/International Society of Urological Pathology consensus classification system. Adv Anat Pathol2002; 9:222-232,.

8. Amin MB, Murphy WM, Reuter VE, et al: Controversies in the pathology of transitional cell carcinomas of the urinary bladder. In Rosen PP, Fechner RE (eds): Reviews of Pathology. Chicago, ASCP Press, 1996, pp 1-39.

9. Amin MB, Patel RM, Oliveira P, et al: Alveolar soft-part sarcoma of the urinary bladder with

urethral recurrence: A unique case with emphasis on differential diagnoses and diagnostic utility of an immunohistochemical panel including TFE3. Am J Surg Pathol2006; 30:1322-1325.

10. Amin MB, Ro JY, el-Sharkawy T, et al: Micropapillary variant of transitional cell carcinoma of the urinary bladder: Histologic pattern resembling ovarian papillary serous carcinoma. Am J Surg Pathol1994; 18:1224-1232.

11. Amling CL: Diagnosis and management of superficial bladder cancer. Curr Probl Cancer 2001; 25:219-278.

12. Anderstrom C, Johansson SL, Pettersson S, Wahlqvist L.Carcinoma of the ureter: a clinicopathologic study of 49 cases. J Urol 1989; 142: 280-283.

13. Anttila S, Hietanen E, Vainio H, et al. Smoking and peripheral type of cancer are related to high levels of pulmonary cytochrome P450IA in lung cancer patients. Int J Cancer 1991; 47: 681: 685.

14. Arge P, Hoover R Kantor A: bladder cancer risks and pipes , cigars and smokeless tobacoo.cancer 1985 :55:901.

15. Augustine A, Hebert JR, Kabat GC, et al: bladder cancer in relation to cigarette smoking.Cancer Res 1988; 48:4405.

16. AugustineA, Hebert JR, Kabat GC et al: bladder cancer in relation to cigarette smoking . Cancer RES 1988; 48: 4405.

17. Bale AE, Brown SJ. Etiology of cancer: cancer genetics. In: Cancer. Principles and practice of oncology, 6th ed. Philadelphia: Lippincott Williams & Wilkins, 2001, pp. 207:217.

18. Battifora H, Eisenstein R, McDonald JH. The human urinary bladder mucosa. An electron

microscopic study. Invest Urol 1964; 12:354:361.

19. Bostwick DG, Mikuz G: Urothelial papillary (exophytic) neoplasms. Virchows Arch 2002: 441:109-116.

20. Brennan P, Bogillot O, Cordier S, Greiser E, Schill W, Vineis P, Lopez- Abente G, Tzonou Chang-Claude J, Bolm-Audorff U, Jockel KH, Donato F, Serra C, Wahrendorf J, Hours M, T'Mannetje A, Kogevinas M, Boffetta P. Cigarette smoking and bladder cancer in men: a pooled analysis of 11 case-control studies. Int J Cancer 2000:86: 289- 294.

21. Bronchud MH, Foote M, Peters WP, Robinson MO (eds). Principles of molecular oncology. Totowa, NJ: Humana Press, 2000.

22. Busch C, Hawes D, Johansson SL, Cote RJ: Pathologic assessment of bladder cancer and pitfalls in staging. In Droller MJ (ed): Bladder Cancer: Current Diagnosis and Treatment. Totowa, NJ, Humana Press, 2001.

23. Cairns P, Proctor AJ, Knowles MA: Loss of heterozygosity at the RB locus is frequent and correlates with muscle invasion in bladder carcinoma. Oncogene1991; 6: 2305-2309.

24. Cancer Incidence and Mortality in Eastern Libya, 2004 Benghazi Cancer Registry

25. Chen KC, Chiang HS, Fang CL: EBER expression of pure urinary bladder lymphoepithelioma- like carcinoma in two unique Asian patients. Urol Int2005: 74:280-282.

26. Cheng L, Darson M, Cheville JC, et al: Urothelial papilloma of the bladder: Clinical and biologic implications. Cancer1999; 86:2098-2101.

27. Cheng L, Jones TD, McCarthy RP, et al: Molecular genetic evidence for a common clonal origin of urinary bladder small cell carcinoma and coexisting urothelial carcinoma. Am J Pathol2005; 166:1533-1539,

28. Cheng L, Neumann RM, Bostwick DG: Papillary urothelial neoplasms of low malignant potential: Clinical and biologic implications. Cancer 1999; 86: 2102-2108.

29. Cheng L, Pan CX, Yang XJ, et al: Small cell carcinoma of the urinarybladder: A clinicopathologic analysis of 64 patients. Cancer 2004; 101:957-962.

30. Choong NW, Quevedo JF, Kaur JS: Small cell carcinoma of the urinary bladder: The Mayo Clinic experience. Cancer2005; 103:1172- 1178.

31. ClavelJ , Corder S , Bocon –GibodL, et al : Tobacco and bladder cancer in males : increased risk for inhalers and smokers of black tobacco. Int JCancer 1989 : 44:605.

32. Clement PB, Young RH. Endocervicosis of the urinary bladder. A report of six cases of a benign mullerian lesion that may mimic adenocarcinoma. Am J Surg Pathol 1992; 16:533, 542.

33. Comiter CV. Endometriosis of the urinary tract. Urol Clin North Am 2002; 29:625, 635.

34. Cordon-Cardo C: Mutations of cell cycle regulators: Biological and clinical implications for human neoplasia. Am J Pathol1995; 147:545-560.

35. Cote R. J., Chatterjee S. J. Molecular determinants of outcome in bladder cancer. Cancer J. Sci. Am., 1999; 5: 2-15.

36. Datta SN, Allen GM, Evans R, Vaughton KC, Lucas MG. Urinary tract ultrasonography in the evaluation of haematuria—a report of over 1,000 cases.Ann R Coll Surg Engl 2002; 84: 203-205.

37. Deisseroth AB, DeVita VT Jr: The cell cycle: Probing new molecular determinants of resistance and sensitivity to cytotoxic agents. Cancer J Sci Am1995; 1:15-21.

38. Denkhaus H, Crone-Munzebrock W, Huland H. Noninvasive ultrasound in detecting and staging bladder carcinoma.Urol Radiol1985 ; 7: 121-131.

39. Desai S, Lim SD, Jimenez RE, Chun T, Keane TE, McKenney JK, Zavala-Pompa A, Cohen C, Young RH, Amin MB. Relationship of cytokeratin 20 and CD44 protein expression with WHO/ISUP grade in pTa and pT1 papillary urothelial neoplasia. Mod Pathol 2000; 13: 1315-1323.

40. Dietrich H, Dietrich B. Ludwig Rehn (1849-1930)—pioneering findings on he aetiology of bladder tumours. World J Urol 2001; 19: 151-153.

41. Dixon JS, Gosling JA. Histology and fine structure of the muscularis mucosae of the human urinary bladder. J Anat 1983; 136(pt 2):265; 271.

42. Eble JN, Epstein JI, Sesterhenn IA: World Health Organization Classification of Tumors. Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs. Lyon, France, IARC Press, 2004.

43. Eble JN, Sauter G, Epstein JE, Sesterhenn IA, eds.World Health Organization Classification of Tumors: Pathology and Genetics. Tumors of the Urinary System and Male Genital Organs.Lyon: IARC; 2000.

44. Elbadawi A. Anatomy and function of the ureteral sheath. J Urol 1972; 107: 224 , 229.

45. El-Bolkainy MN, Mokhtar NM, Ghoneim MA, Hussein MH: The impact of schistosomiasis on the pathology of bladder carcinoma. Cancer 1981; 48:2643-2648.

46. Epstein JI, Amin MB, Reuter VR, Mostofi FK: The World Health Organization/International Society of Urological Pathology consensus classification of urothelial (transitional cell) neoplasms of the urinary bladder: Bladder Consensus Conference Committee. Am J Surg Pathol 1998; 22: 1435-1448.

47. Esrig D, Elmajian D, Groshen S, et al: Accumulation of nuclear p53 and tumor progression in bladder cancer. N Engl J Med1994; 331: 1259-1264.

48. Esrig, D. , C. H. I. I. I. Spruck , and P. W. Nichols . et al. p53 nuclear protein accumulation correlates with mutations in the p53 gene, tumor grade, and stage in bladder cancer. Am J Pathol 1993; 143: 1389–1397.

49. Fawcett DW, Raviola E, eds. Bloom and Fawcett: A Textbook of Histology. 12th ed. New York: Chapman & Hall; 1994.

50. Fawcett DW. Bloom and Fawcett: A Textbook of Histology. 11th ed. Philadelphia: WB Saunders; 1986.

51. Fellinger EJ, Garin-Chesa P, Su SL, et al: Biochemical and genetic characterization of the HBA71 Ewing's sarcoma cell surface antigen. Cancer Res 1991; 51:336-340.

52. Fellinger EJ, Garin-Chesa P, Triche TJ, et al: Immunohistochemical analysis of Ewing's sarcoma cell surface antigen p30/32MIC2. Am J Pathol 1991; 139:317- 325.

53. Fine SW, Humphrey PA, Dehner LP, et al: Urothelial neoplasms in patients 20 years or younger: A clinicopathological analysis using the World Health Organization 2004 bladder consensus classification. J Urol 2005; 174:1976-1980.

54. Fletcher TF, Bradley WE. Neuroanatomy of the bladder-urethra. J Urol 1978;119:153 ,160

55. Foster CS, Ross JS, eds. Pathology of the Urinary Bladder.Philadelphia: Saunders; 2004.

56. Fujii Y, Kawakami S, Koga F, et al: Long-term outcome of bladder papillary urothelial neoplasms of low malignant potential. BJU Int 2003; 92:559-562.

57. Fujii Y, Kawakami S, Koga F, et al: Long-term outcome of bladder papillary urothelial

neoplasms of low malignant potential. BJU Int 2003; 92:559-562.

58. Fujimoto, K. , Y. Yamada , and E. Okajima . et al. Frequent association of p53 gene mutation in invasive bladder cancer. Cancer Res 1992. 52:1393–1398.

59. Gan J, Skipper PL , Gago- Dominguez M et al :Alkylaniline – hemoglobin adduct and risk of non-smoking-related bladder cancer.J Natl Cancer Inst 2004;96:1425-1431.

60. Gattuso P, Reddy V, Daved O, Spitz D ,Hebar M differential diagnosis in surgical Pathology ,Saunders Elsevier ,2nd edtion,2010. 136- Virchow R: Cellular pathology as based upon physiological and pathological histology. (Translated from the second edition of the original by F. Chance, 1860.) In Bogdonoff MD, Good RA, McGovern JP (eds): The Classics of Medicine Library, Special Edition, 1978. Birmingham, UK, The Classics of Medicine Library, Division of Gryphon Editions, 1978, p 468.

61. Goddard JC, Sutton CD, Jones JL, O'Byrne KJ, Kockelbergh RC. Reduced thrombospondin-1 at presentation predicts disease progression in superficial bladder cancer. Eur Urol 2002; 42: 464-468.

62. Gonzalez-Zulueta M, Ruppert JM, Tokino K, et al: Microsatellite instability in bladder cancer. Cancer Res 1993; 53:5620-5623.

63. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations with p53 tumor suppressor gene: Clues to cancer etiology and molecular pathogenesis. Cancer Res. with neoadjuvant MVAC. J Clin Oncol 1995; 13: 1384.

64. Grignon DJ, Ro JY, Ayala AG, et al: Primary adenocarcinoma of the urinary bladder: A clinicopathologic analysis of 72 cases. Cancer 1991; 67:2165-2172.

65. Grignon DJ, Ro JY, Ayala AG, et al: Primary adenocarcinoma of theurinary bladder: A clinicopathologic analysis of 72 cases. Cancer1991; 67: 2165- 2172.

66. Grignon DJ, Ro JY, Ayala AG, Johnson DE: Primary signet-ring cell carcinoma of the urinary bladder. Am J Clin Pathol 1991; 95:13- 20.

.

67. Grossman HB, Liebert M, Antelo M, et al: p53 and RB expression predict progression in T1 bladder cancer. Clin Cancer Res1998; 4: 829-834.

68. Grossman HB, Schmitz-Drager B, Fradet Y, Tribukait B: Use of markers in defining urothelial premalignant and malignant conditions. Scand J Urol Nephrol Suppl 2000; 205:94-104.

69. Gulley ML, Amin MB, Nicholls JM, et al: Epstein-Barr virus is detected in undifferentiated nasopharyngeal carcinoma but not in lymphoepithelioma-like carcinoma of the urinary bladder. HumPathol, 1995; 26: 1207-1214.

70. Haleblian GE, Skinner EC, Dickinson MG, Lieskovsky G, Boyd SD, Skinner DG. Hydronephrosis as a prognostic indicator in bladder cancer patients. J Urol 1998; 160: 2011-2014.

71. Harano, H. C. Wang, J. Gao, and T. Uchida. p53 tumor suppressor gene mutation and prognosis in 105 cases of bladder cancer. The relationship between mutation of the p53 gene with clinicopathologic features and smoking. Jpn J Urol 1999; 90:487–495.

72. Hartge P, Harvey EB, Linehan WM, et al: Unexplained excess risk of bladder cancer in men. J Natl Cancer Inst, 1990; 82: 1636-1640.

73. Hartge P, Hoover R, West DW, Lyon JL. Coffee drinking and risk of bladder cancer. J Natl Cancer Inst 1983; 70: 1021- 1026.

74. Hasui Y, Osada Y, Kitada S, Nishi S. Significance of invasion to the muscularis mucosae on the progression of superficial bladder cancer. Urology1994; 43:782-786.

75. Helpap B,Ayala AG, Grignon DJ etal: metastatic tumors and secondary extension in urinary

bladder.In eble JN, Sauter G , Epstein JI,SesterhennIA:WHO classification of Tumors:Pathology &Genatics:Tumors of the urinary system and Male Genital organs.Lyon , france ,IARC Prees 2004;148-149.

76. Hicks RM. The function of the golgi complex in transitional epithelium. Synthesis of the thick cell membrane. J Cell Biol 1966; 30:623: 643.

77. Histology for Pathologists, Lippincott Williams & Wilkins, 3rd EditionCopyright 2007.

78. Holmang S, Andius P, Hedelin H, Wester K, Busch C, Johansson SL. Stage progression in Ta papillary urothelial tumors: relationship to grade, immunohistochemical expression of tumor markers, mitotic frequency and DNA ploidy. J Urol 2001; 165: 1124-1128.

79. Holmang S, Hedelin H, Anderstrom C, Holmberg E, Busch C, Johansson SL.Recurrence and progression in low grade papillary urothelial tumors. J Urol1999; 162: 702-707.

80. Holmang S, Johansson SL. Stage Ta-T1 bladder cancer: the relationship between findings at first followup cystoscopy and subsequent recurrence and progression. J Urol 2002; 167: 1634-1637.

81. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Some Drinking-water Disinfectants and Contaminants, including Arsenic. IARC Press: Lyon (in press). 2004.

82. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Tobacco smoke and involuntary smoking. IARC Press: Lyon (in press), 2004.

83. Ikegami H, Iwasaki H, Ohjimi Y, et al: Sarcomatoid carcinoma of the urinary bladder: A clinicopathologic and immunohistochemical analysis of 14 patients. Hum Pathol, 2000; ,31: 332-340.

84. Jemal A, Siegel R, Ward E, et al: Cancer statistics, 2007. CA Cancer J Clin, 2007; 57: 43-66.

85. Jequier S, Rousseau O. Sonographic measurements of the normal bladder wall in children. AJR Am J Roentgenol 1987; 149: 563 ,566.

86. Jones EC, Young RH: Nonneoplastic and neoplastic spindle cell proliferations and mixed tumors of the urinary bladder. J Urol Pathol, 1994; 2:105-134.

87. Kantor AF, Hartage P,Hoover RN et al: Urinary tract infection and risk of bladder cancer. Am J Epidemiol 1984; 119: 510.

88. Kausch I, Bohle A . Molecular aspects of bladder cancer III. Prognostic markers of bladder cancer. Eur Urol2002; 41: 15-29.

89. King M-C. Genetic analysis of cancer in families. Cancer Surv 1990; 9: 417:433.

90. Kissane JM. Development and structure of the urogenital system. In: Murphy WM, ed. Urological Pathology. Philadelphia: WB Saunders: 1989; 1, 33.

91. Koraitim M, Kamal B, Metwalli N, Zaky Y. Transurethral ultrasonographic assessment of bladder carcinoma: its value and limitation. J Urol 1995 ; 154: 375-378.

92. Koss LG. The asymmetric unit membranes of the epithelium of the urinary bladder of the rat. An electron microscopic study of a mechanism of epithelial maturation and function. Lab Invest 1969; 21:154:168.

93. Koss LG. Tumors of the urinary bladder. In: Atlas of Tumor Pathology. 2nd series, fascicle 11. Washington, DC: Armed Forces Institute of Pathology; 1975.

94. Koss LG: Mapping of the urinary bladder: Its impact on the concepts of bladder cancer. Hum

Pathol, 1979; 10: 533-548.

95. Kunju LP, Lee CT, Montie J, Shah RB: Utility of cytokeratin 20 and Ki-67 as markers of urothelial dysplasia. Pathol Int, 2005; 55: 248-254.

96. Leroy X, Leteurtre E, De La Taille A, et al: Microcystic transitional cell carcinoma: A report of 2 cases arising in the renal pelvis. Arch Pathol Lab Med, 2002; 126:859-861.

97. LIANG CHENG, DAVID G. BOSTWICK; Essentials of anatomic pathology 2002.

98. Liedberg F, Chebil G, Davidsson T, et al: The nested variant of urothelial carcinoma: A rare but important bladder neoplasm withaggressive behavior. Three case reports and a review of the literature. Urol Oncol, 2003; 21: 7-9.

99. Lipponen P, Eskelinen M. Expression of epidermal growth factor receptor in bladder cancer as related to established prognostic factors, oncoprotein (c-erbB-2, p53) expression and long-term prognosis. Br J Cancer. 1994; 69:1120–5.

100. Lipponen PK: Over-expression of p53 nuclear oncoprotein in transitional-cell bladder cancer and its prognostic value. Int J Cancer, 1993; 53:365-370.

101. Liu JB, Bagley DH, Conlin MJ, Merton DA, Alexander AA, Goldberg BB. Endoluminal sonographic evaluation of ureteral and renal pelvic neoplasms. J Ultrasound Med, 1997; 16: 515-521.

102. LucasSB:Squamous cell carcinoma of the bladder and schistosommiasis. East Afr med J 1982; 59: 345.

103. Mahadevia PS, Koss LG, Tar IJ. Prostatic involvement in bladder cancer. Prostate mapping in 20 cystoprostatectomy specimens. Cancer 1986; 58:2096- 2102.

104. Malmstrom PU, Busch C, Norlen BJ. Recurrence, progression and survival in bladder cancer. A retrospective analysis of 232 patients with greater than or equal to 5-year follow-up. Scand

J Urol Nephrol1987; 21: 185-195.

105. Markl ID, Jones PA: Presence and location of TP53 mutation determines pattern of CDKN2A/ARF pathway inactivation in bladder cancer. Cancer Res, 1998; 58: 5348-5353.

106. McCredie M, Ford JM, Taylor JS, Stewart JH. Analgesics and cancer of the renal pelvis in New South Wales. Cancer1982; 49: 2617-2625.

107. McKenney JK, Desai S, Cohen C, Amin MB: Discriminatory immunohistochemical staining of urothelial carcinoma in situ and non-neoplastic urothelium: an analysis of cytokeratin 20,p53, and CD44 antigens. Am J Surg Pathol, 2001; 25:1074-1078.

108. McKenney JK, Gomez JA, Desai S, et al: Morphologic expressions of urothelial carcinoma in situ: A detailed evaluation of its histologic patterns with emphasis on carcinoma in situ with microinvasion. Am J Surg Pathol, 2001; 25:356-362.

109. Messing EM, Vaillancourt A. Hematuria screening for bladder cancer. J Occup Med, 1990; 32: 838-845

110. Mihatsch MJ, Knusli C. Phenacetin abuse and malignant tumors. An autopsy study covering 25 years (1953-1977).Klin Wochenschr, 1982; 60: 1339-1349.

111. Mills, Stacey E.; Carter, Darryl; Greenson, Joel K.; Oberman, Harold A.; Reuter, Victor E.; Stoler, Mark H. Sternberg's Diagnostic Surgical Pathology, 4th Edition Copyright ©2004 Lippincott Williams & Wilkins.

112. Mitra AP, Datar RH, Cote RJ: Molecular pathways in invasive bladder cancer: New insights into mechanisms, progression, and target identification. J Clin Oncol, 2006; 24:5552-5564.

113. Moore K. The urinary system. In: Moore K, ed. The Developing Human: Clinically Oriented Embryology. Philadelphia: WB Saunders; 1982.

114. Moore KL, Dalley AF. Clinically Oriented Anatomy. 5th ed. Baltimore: Lippincott Williams

& Wilkins; 2005.

115. Moore KL. The pelvis and perineum. In: Moore KL, ed. Clinically Oriented Anatomy. 2nd ed. Baltimore: Williams & Wilkins; 1985.

116. Morrison AS: Advances in the etiology of urothelial cancer. Urol Clin North Am 1984; 11:557.

117. Mostofi FK, Davis CJ, Sesterhenn IA: Pathology of tumors of the urinary tract. In Skinner DG, Lieskovsky G (eds): Diagnosis and Management of Genitourinary Cancer. Philadelphia, WB Saunders, 1988, pp 83-117.

118. Mostofi FK, Thomson RV, Dean AL Jr: Mucous adenocarcinoma of the urinary bladder. Cancer1955; 8:741-758.

119. Mufti GR, Singh M: Value of random mucosal biopsies in the management of superficial bladder cancer. Eur Urol, 1992; 22:288-293.

120. Murphy WM, Busch C, Algaba F: Intraepithelial lesions of urinary bladder: Morphologic considerations. Scand J Urol Nephrol Suppl, 2000; 205:67-81.

121. Murphy WM, Grignon DJ, Perlman EJ. Atlas of Tumor Pathology.Tumors of the Kidney, Bladder, and Related UrinaryStructures. 4th series. Washington, DC: Armed Forces Institute of Pathology; 2004.

122. N Ibrahim, A Elzagheid, H El-Hashmi, K Syrjänen, and S Alhakim The Potential Value of EGFR and P53 Immunostaining in Tumors of the Urinary Bladder, 2009.

123. Nagy GK, Friedell GHY: Urinary bladder. In Henson DE, Albores-Saavedra J (eds): Pathology of Incipient Neoplasia, 2nd ed. Philadelphia, WB Saunders, 1993.

124. Negri E, La Vecchia C (2001). Epidemiology and prevention of bladder cancer. Eur J Cancer Prev 10: 7-14.

125. Newman J, Antonakopoulos GN. The fine structure of the human fetal urinary bladder. Development and maturation. A light, transmission and scanning electron microscopic study. J Anat 1989; 166:135:150.

126. Olgac S, Mazumdar M, Dalbagni G, Reuter VE. Urothelial carcinoma of the renal pelvis: a clinicopathologic study of 130 cases. Am J Surg Pathol 2004; 28:154,1552.

127. Oliva E, Amin MB, Jimenez R, Young RH: Clear cell carcinoma of the urinary bladder: A report and comparison of four tumors of mullerian origin and nine of probable urothelial origin with discussion of histogenesis and diagnostic problems. Am J Surg Pathol 2002; 26:190- 197.

128. Orlow I, Lianes P, Lacombe L, et al: Chromosome 9 allelic losses and microsatellite alterations in human bladder tumors. Cancer Res1994; 54:2848-2851.

129. Pashos CL, Botteman MF, Laskin BL, Redaelli A. Bladder cancer:epidemiology, diagnosis, and management. Pract 2002; 10: 311-322.

130. Paz A, Rath-Wolfson L, Lask D, et al: The clinical and histological features of transitional cell carcinoma of the bladder with microcysts: Analysis of 12 cases. Br J Urol1997; 79:722-725.

131. Pennisi E. Behind the scenes of gene expression. Science 2001; 293: 1064:1067.

132. Pich A, Chiusa L, Formiconi A, Galliano D, Bortolin P, Navone R.Biologic differences between noninvasive papillary urothelial neoplasms of low malignant potential and low-grade (grade 1) papillary carcinomas of the bladder. Am J Surg Pathol2001; 25: 1528-1533.

133. Politano VA. Ureterovesical junction. J Urol 1972; 107:239 ,242.
134. Ponder BAJ. Inherited cancer syndromes. In: Carney D, Sikora K, eds. Genes and cancer. Chichester: John Wiley & Sons, 1990, pp. 99:106.

135. Ramos-Vara, JA. "Technical Aspects of Immunohistochemistry". Vet Pathol 2005; 42 (4): 405–426).
136. Riede / Werner, Color Atlas of Pathology, 2004.

137. Ro JY, Ayala AG, el-Naggar A, Wishnow KI. Seminal vesicle involvement by in situ and invasive transitional cell carcinoma of the bladder. Am J Surg Pathol 1987; 11:951-958.

138. Ro JY, Ayala AG, el-Naggar A. Muscularis mucosa of urinary bladder. Importance for staging and treatment. Am J Surg Pathol 1987; 11: 668 -673.

139. Robbins&cotran,pathological basis of diseas ,5th edtion 2007.

140. Robertson AJ, Beck JS, Burnett RA, et al: Observer variability in histopathological reporting of transitional cell carcinoma and epithelial dysplasia in bladders. J Clin Pathol1990; 43: 17-21,.

141. Rodriguez-Alonso A, Pita-Fernandez S, Gonzalez-Carrero J, Nogueira-March JL.Multivariate analysis of survival, recurrence, progression and development of mestastasis in T1 and T2a transitional cell bladder carcinoma. Cancer 2002; 94: 1677-1684.

142. Rubin's Pathology : Clinicopathologic Foundations of Medicine, 5th Edition Copyright 2008 Lippincott Williams & Wilkins

143. Russo P, Brady MS, Conlon K, et al: Adult urological sarcoma. J Urol 147:1032-1036, 1992.
144. Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. Curr Opin Cell Biol 2001; 13: 332-7.

145. Samaratunga H, Khoo K: Micropapillary variant of urothelial carcinoma of the urinary bladder: A clinicopathological and immunohistochemical study. Histopathology2004; 45:55-64 .

146. Sarkis AS, Bajorin DF, Reuter VE, Herr HV, Netto G, Zhang ZF, . Prognostic value of p53 nuclear over expression in patients with invasive bladder cancer treated with neoadjuvant MVAC. J Clin Oncol 1995; 13: 1384.

147. Sarkis AS, Dalbagni G, Cordon-Cardo C, Zhang ZF, Sheinfeld J, Fair WR, Herr HW,Reuter VE. Nuclear overexpression of p53 protein in transitional cell bladder carcinoma:a marker for disease progression. JNatl Cancer Inst 1993; 85: 53-59.

148. Schmitz-Drager BJ, Goebell PJ, Ebert T, Fradet Y. p53 immunohistochemistry as a prognostic marker in bladder cancer. Playground for urology scientists? Eur Urol 2000; 38: 691- 699.

149. Shehata R. A comparative study of the urinary bladder and the intramural portion of the ureter. Acta Anat (Basel) 1977; 98:380 ,395.

150. Skinner DG: Current perspectives in the management of high-grade invasive bladder cancer. Cancer 45(Suppl): 1980; 1866-1874.

151. Smith AH, Goycolea M, Haque R, Biggs ML. Marked increase in bladder and lung cancer mortality in a region of Northern Chile due to arsenic in drinking water. Am J Epidemiol 1998; 147: 660-669.

152. Soini, Y. , D. Turpeenniemi-Hujanen , and D. Kamel . et al. p53 immunohistochemistry in transitional cell carcinoma and dysplasia of the urinary bladder correlates with disease progression. Br J Cancer 1993. 68:1029–1034.

153. Stein JP, Lieskovsky G, Cote R, et al: Radical cystectomy in the treatment of invasive bladder cancer: Long-term results in 1,054 patients. J Clin Oncol 2001; 19:666-675.

154. Sunanda J. Chatterjee, Ram Datar, David Youssefzadeh, Ben George, Peter J. Goebell, John P. Stein, Lillian Young, Shan-Rong Shi, Conway Gee, Susan Groshen, Donald G. Skinner, Richard J. Cote ,From the Departments of Pathology, Urology, and Preventive Medicine, University of Southern California Keck School of Medicine; and University of Southern California/Kenneth Norris Comprehensive Cancer Center, Los Angeles, CA 2004.

155. Talbert ML, Young RH: Carcinomas of the urinary bladder with deceptively benign-appearing foci: A report of three cases. Am J Surg Pathol 1989; 13:374-381.

156. Tanagho E. Anatomy of the lower urinary tract. In: Campbell MF and Walsh PC, eds. Campbell's Urology. 6th ed. Philadelphia: WB Saunders; 1992.

157. Tanagho EA, Meyers FH, Smith DR. The trigone: anatomical and physiological considerations. I. In relation to the ureterovesical junction. J Urol 1968; 100:623,632.

158. Tanagho EA, Smith DR, Meyers FH. The trigone: anatomical and physiological considerations. II. In relation to the bladder neck. J Urol 1968; 100:633,639.

159. Tanagho EA, Smith DR. The anatomy and function of the bladder neck. Br J Urol 1966; 38:54, 71.

160. Tanguay C, Harvey I, Houde M, Srigley JR, Tetu B. Leiomyosarcoma of urinary bladder following cyclophosphamide therapy: report of two cases. Mod Pathol 2003; 16: 512-51

161. Tawfik HN: Carcinoma of the urinary bladder associated with schistosomiasisin Egypt: The possible causal relationship. Princess Takamatsu Symp1987; 18:197-209

162. Tricker AR ,Mostafa MH,Spiegelhalder B, et al:Urinary execretion of nitrate,nitrite andN-Nitrose compounds in schistosommiasis and bilharzial bladder cancer patients. Carcinogene Sis1989; 10: 547.

163. Urist MJ, di Como CJ, Lu ML, Charytonowicz E, Verbel D, Crum CP, Ince TA, McKeon FD, Cordon-Cardo C. Loss of p63 expression is associated with tumor progression in bladder cancer. Am J Pathol 2002; 161: 1199- 1206.

164. Utz DC, Farrow GM, Rife CC, Segura JW, Zincke H. Carcinoma in situ of the bladder. Cancer 1980; 45(suppl 7):1842-1848.

165. Van Oers JM, Adam C, Denzinger S, et al: Chromosome 9 deletions are more frequent than FGFR3 mutations in flat urothelial hyperplasias of the bladder. Int J Cancer 2006; 119:1212-1215.

166. Van Rhijn BW, Montironi R, Zwarthoff EC, et al: Frequent FGFR3 mutations in urothelial papilloma. J Pathol 2002; 198: 245-251.

167. Vardar E, Gunlusoy B, Minareci S, Postaci H, Ayder AR. Evaluation of p53 nuclear accumulation in low- and high-grade (WHO/ISUP classification) transitional papillary carcinomas of the bladder for tumor recurrence and progression. Urol Int. 2006 ;77:27–33

168. Vogelstein B, Kinzler KW (eds). The genetic basis of human cancer. New York: McGraw-Hill, 1998.

169. Vogelstein, B. and K. W. Kinzler. P53 function and dysfunction. Cell 1992. 70:523–526.

170. WaterhouseJ, Muir C, Shanmugartanam K, et al: Cancer incidence in five continents, lyon, International Agency for research on cancer, 1982.

171. Weiss L. Cell and Tissue Biology: A Textbook of Histology. 6th ed. Baltimore: Urban & Schwarzenberg; 1988.

172. Wilson TG, Pritchett TR, Lieskovsky G, et al: Primary adenocarcinoma of bladder. Urology

1991; 38:223-226.

173. Woolcott CG, King WD, Marrett LD. Coffee and tea consumption and cancers of the bladder, colon and rectum. Eur J Cancer Prev 2002; 11: 137-145.
174. Xiao GQ, Savage SJ, Gribetz ME, et al: The nested variant of urothelial carcinoma: Clinicopathology of 2 cases. Arch Pathol Lab Med 2003; 127:333-336.

175. Yin H, He Q, Li T, Leong AS: Cytokeratin 20 and Ki-67 to distinguish carcinoma in situ from flat non-neoplastic urothelium. Appl Immunohistochem Mol Morphol2006; 14:260-265.

176. Yoon DS, Li L, Zhang RD, Kram A, Ro JY, Johnston D, Grossman HB, Scherer S, Czerniak B. Genetic mapping and DNA sequence-based analysis of deleted regions on chromosome 16 involved in progression of bladder cancer from occult preneoplastic conditions to invasive disease. Oncogene 2001; 20: 5005-5014.

177. Young RH, Clement PB. Mullerianosis of the urinary bladder. Mod Pathol 1996; 9:731-737.

178. Young RH, Eble JN: Unusual forms of carcinoma of the urinary bladder. Hum Pathol 1991; 22:948-965.

179. Young RH, Wick MR, Mills SE: Sarcomatoid carcinoma of the urinary bladder: A clinicopathologic analysis of 12 cases and review of the literature. Am J Clin Pathol1988; 90: 653-661.

180. Zaghloul MS, Nouh A, Nazmy M, et al: Long-term results of primary adenocarcinoma of the urinary bladder: A report on 192 patients. Urol Oncol 2006; 24: 13-20,.

181. Zincke H, Utz DC, Farrow GM: Review of Mayo Clinic experience with carcinoma in situ. Urology1985; 26(Suppl):39-46.

الملخص العربى

البروتين 53:-

المعروف أيضا ببروتين مثبط الورم 53 , هو جين مثبط للأورام في البشر، وهو مشفرة بواسطة المورثة TP53 في الصبغي 17 لدى البشر .للبروتين 53 دور مهم في الكائنات متعددة الخلايا حيث يقوم في تنظيم دور ة الخلية وبالتالي يلعب دور رئيسي في كبح الأورام والوقاية من السرطان. وبالتالي وصف Tp53 بأنه "حامي الجينوم"، و"الحارس" ، وذلك مشيراً إلى دوره في الحفاظ على استقرار المعلومات الوراثيةمن خلال منع الطفرات الوراثية.

يشير الاسم Tp53إلى كتلة البروتين الجزيئية باعتباره 53 كيلو دالتون عن طريق حسابه بواسطة إس دي إس بايج .إلا أن الكتلة الفعلية للبروتين تبلغ 43.7 وذلك عن طريق حساب مخلفات الأحماض الأمينية. هذا الاختلاف يرجع إلى العدد الكبير من البرولين في البروتين والتي تبطئ من الهجرة في إس دي إس بايج مما يجعلها تبدو أثقل مما هو عليه بالفعل [5]. هذا التأثير لوحظ مع p53 من مختلف الأنواع، بما في ذلك البشر والقوارض والضفادع، والأسماك.

سرطان المثانة:-

سرطان المثانة هو إصابة سرطانية لمنطقة المثانة . يبدأ سرطان المثانة في معظم الأحيان بإصابة الخ لايا المبطنة للمثانة من الداخل. وعادة ما يصيب كبار السن، إلا أن هذا لا يعني استثناء أي سن من الاصابة.

سرطان المثانة هو خامس أكثر أنواع السرطانات شيوعا بين الرجال والثامن الأكثر شيوعا لدى النساء حسب معهد السرطان الوطني الأمريكى ، ويشكل رابع أكثر أنواع السرطانات شيوعا بين الرجال في بريطانيا و في عام 1996 أكثر من 300000 حالة جديدة تم تشخيصها في جميع أنحاء العالم . يتم تشخيص الغالبية العظمى من سرطان المثانة في مراحله المبكرة، حيث يكون قابلا للعلاج بصورة جيدة جدا. ولكن، إصابة سرطان المثانة تتميز بالتكرر حتى لو تم العلاج ضمن مرحلة مبكرة، ولهذا السبب، يتوجب على الناجين من مرض سرطان المثانة أن يتابعوا الاختبارات ومراقبة حالتهم لعدة سنوات بعد العلاج. فدرت عدد الاصابات الجديدة بهذا المرض في الولايات المتحدة لعام 2008 بحوالي 67000 إصابة، كان عدد الوفيات ضمنها 14000 حالة .

لا يوجد سبب علمي واضح لتفسير تكون سرطان المثانة، إلا أنه تم التعرف على عدد من المواد المسرطنة التي يمكن اعتبارها ضمن قائمة الأسباب المحتملة، إهم هذه الأسباب هو دخان السجائر. يتركز البحث العلمي على فهم الظروف التي تؤدي إلى تغير التركيبة الوراثية للخلايا، مما يسبب تكاثر الخلايا غير الطبيعية. العوامل التالية تؤدي إلى زيادة مخاطر تطوير سرطان المثانة .

مسببات سرطان المثانة البولية:-

:

1- **التدخين** : التدخين هو أهم عامل في زيادة خطر سرطان المثانة .المدخنون معرضون بما يفوق المرتين لخطر تطوير سرطان المثانة مقارنة مع غير المدخنين.

2- التعرض لمواد كيميائية في مكان العمل : الأفراد الذين يتعاملون بانتظام مع بعض المواد الكيميائية أو في بعض الصناعات لديهم احتمال الاصابة بسرطان المثانة مرتفع مقارنة مع الاخرين. المواد الكيميائية العضوية الأمينات العطرية متبطة بصورة عالية مع سرطان المثانة .هذه المواد الكيميائية مستخدمة وبكثرة ضمن صناعة الاصباغ. الصناعات الأخرى المرتبطة بسرطان المثانة تشمل على دباغة الجلود والصناعات المطاطية، والمنسوجات، وأصباغ الشعر، صناعة مواد الالوان، ومواد الطباعة.

أهم هذه المواد هي :

1-أصباغ الأنيلين

2-2- نافثيلامين

3-4 - أمينزوبايفينيل :

4- البنزيدين

3- **ا لنظام الغذائي :** الافراد الذين يتناولون نظام غذائي يشمل كميات كبيرة من اللحوم المقلية والدهون الحيوانية هم أكثر عرضة لتعرض للسرطان المثانة.

العوامل المساعدة على تكون سرطان المثانة

1-علاج مرض سرطاني أخر.

2 - العلاج الاشعاعي للامراض السرطانية ضمن منطقة الحوض، مثل سرطان عنق الرحم، و سرطان البروستاتا، سرطان الكلى، وسرطان قناة فالوب وسرطان الخصية يزيد من خطر الاصابة بسرطان المثانة.

3- العلاج الكيميائي بعقار سِيكْلُوفُسْفاميد :يزيد من خطر سرطان المثانة.

الاعراض:-

كثيرا ما يتأخر ظهور أعراض سرطان المثانة حتى يصل إلى مرحلة متقدمة حيث يصبح علاجها أصعب. ولذلك، فمن المناسب أن يقوم الشخص المعرض للمسببات وتتوفر لديه العوامل المساعدة (حتى وإن لم يعانوا من أي أعراض) باختبار الفرز. وهو اختبار لفحص السرطان لدى الافراد الذين لم يسبق لهم أن أصيبوا بالمرض.

وأهم أعراض مرض سرطان المثانة تشمل ما يلى:

1 - البيلة الدموية وهي وجود الدم في البول:-

الأفراد الذين يمكنهم رؤية الدم في البول، خاصة كبار السن من الذكور الذين يدخنون، يجب أن يعتبروا أن لديهم احتمال إصابة عال جدا بسرطان المثانة حتى يثبت العكس. ، فالدم في البول هو عادة أول علامة تحذير من سرطان المثانة. إلا أن الدم غالبا ما يكون غير مرئيا. وهذا ما يسمى البيلة الدموية المجهرية، إلا أنه قابل للكشف بواسطة اختبار بول بسيط. وفي بعض الحالات، يوجد كمية من الدم ضمن البول ما يكفي لتغيير لون البول بشكل ملحوظ. فيصبح لون البول برتقاليا أو قرنفلي، أو قد يكون أحمر فاتحا مع أو بدون جلطات ظاهرة.

2- إحساس بالحرق الحرق أو الألم أثناء التبول دون وجود أي مؤشر على عدوى أو التهاب ضمن المسالك البولية.

3- تغيير في عادات المثانة، مثل الاضط رار إلى التبول أكثر من المعتاد أو الشعور القوي بالحاجة إلى التبول دون خروج كمية بول كبيرة.

ظهور هذه الاعراض ليس دليلا على الاصابه بسرطان المثانة إلا أنه كافي لدفع الشخص لمراجعة الطبيب.

عند وصول السرطان إلى مرحلة متقدمة أكثر، تظهر أعراض إضافية:

4- فقدان الوزن

5- فقدان الشهية

6 - ألم في العظام أو المستقيم، الشرج أو منطقة الحوض .

.

انواع سرطان المثانة:-

معظم حالات سرطان المثانة هي ظهارية المنشأ، وتشكل سرطانة الخلايا الانتقالية 90% منها والباقي يشكلها كل من السرطانة الغدية والسرطانة حرشفية الخلايا . من الأنواع النادرة الأخرى السرطانة صغيرة الخلايا واللمفوما والغرن.

التصنيف الباثولوجى:- (حسب منظمة الصحة العالمية 1973)

- و(أ) ورم حليمى غير منتشر.
- و(1) منتشر ولكن لم يصل بعد الى طبقة عضلات المثانة.
- و (2) منتشر فى الطبقة العضلية.
- و(3) منتشرلما بعد العضلة الى الدهون خارج المثانة.
- و(4) منتشر فى البئية المحيطة مثل البروستاتا , عنق الرحم او جدار الحوض

مراحل سرطان المثانة

يعتير تحديد السرطان في أي مرحلة جزءاً مهما في علاج السرطان السطحي أو سرطان في موقعه الأصلي، هذا يحدث فقط على سطح البطانة الداخلية للمثانة، وإيجاد السرطان في هذه المرحلة يمنح للمريض أمل أكبر في تلقي علاج ناجح.

- المرحلة 0 : السرطان في المرحلة الأولى يحدث في بطانة المثانة الداخلية.
- المرحلة الاولى : الخلايا السرطانية انتشرت الى الطبقة بعد البطانة الداخلية للمثانة البولية ولكن ليسلعضلات المثانة البولية.
- المرحلة الثانية : الخلايا السرطانية انتشرت الى عضلات جدار المثانة ولكن ليس الى النسيج الده نى المحيط بالمثانة البولية.
- المرحلة الثالثة الخلايا السرطانية انتشرت الى النسيج الدهنى المحيط بالمثانة البولية والى غدة البروستاتا او المهبل او عنق الرحم , ولكن ليس للغدد اللمفاوية او اى من الاعضاء الاخرى.
- المرحلة الرابعة للسرطان وفي هذه المرحلة، تنتشر الخلايا السرطانية إلى العقد اللمفاوية والأعضاء الأخرى، مثل الرئتين أو العظام والكبد.