INTRODUCTION

Bladder cancer is the fourth most common malignancy, in men and the ninth most common in women. It is the eighth leading cause of death in men ***(Edwards et al., 2010).***

The bladder is a common site for cancer development in urinary tract ***(Bangalore et al., 2006).***

At the time of diagnosis, ~80% of urothelial bladder carcinomas are superficial and 20% of them are invasive. Tumors that invade the deep muscle layer of the bladder are assigned stage T2, while T3 and T4 lesions invade the perivesical tissue and local structures respectively ***(Von der Maase et al., 2005).***

In Egyptian males, it may be worthwhile to mention that the urinary bladder cancer is the most common malignancy and has been attributed to schistosomiasis that is linked with squamous cell carcinoma. Recently, incidence of transitional cell carcinoma has been increasing, whereas squamous cell carcinoma has declined ***(Fedewa et al., 2009).***

Approximately 10-15% of superficial or locally invasive (pTa/pT1) tumours progress to muscle invasion, and this risk is dependent on tumour stage and grade. For example, well differentiated (grade 1) tumours progress in only 2% of cases

whereas poorly differentiated (grade 3) tumours progress in up to 20% of cases. However, stage and grade are the subject of 50% inter- and intraobserver variation ***(Van der Meijden et al., 2000).***

Therefore, more accurate prognostic factors are desirable, and genetic markers might fulfill this role ***(Reznikoff et al., 2000)***.

The Her2/neu oncogene is located on chromosome 17q11-21 and encodes for a tyrosine kinase trans-membrane growth factor receptor. Activation of the Her2/neu receptor, following autophosphorylation of the tyrosine kinase residues results in the activation of cascade of intracellular proteins. Ultimately, the mitotic activity and metastatic potential of the cell increases ***(Underwood et al., 1995; Tzahar et al., 1996 and Olayioye et al., 2000).***

Her2/neu expression in bladder carcinoma is variable between different studies ranging between 9 to 81% ***(Sato et al., 1992; Mellon et al., 1996; Chow et al., 2001; Gandour-Edwards et al., 2002 and Wester et al., 2002).***

It has been previously reported that 28% of primary bladder cancers over-express Her2/neu by immunohistochemistry (IHC) and that primary tumor over-expression consistently predicts over-expression in a distant or regional metastatic site ***(Jimenez et al., 2001).*** However, 45%

of Her2-negative primary tumors may show over-expression in their corresponding metastasis. These data suggested that Her2/neu might play a role in the biological progression of bladder cancer and the development of metastatic disease.

AIM OF THE WORK

The aim of this study is to evaluate the expression of the Her2/neu oncoprotein in Urothelial carcinoma and to correlate immunoreactivity of the tumor cells regarding intensity and distribution with clinical data including age, gender, grade and stage of the tumor.

ANATOMICAL HINTS

The bladder is a hollow viscus with the shape of a four-sided inverted pyramid when empty and of a rounded structure when distended ***(Rosai et al., 2011).*** The bladder is a muscular organ with two main functions:

- Low-pressure storage of urine
- High-pressure expulsion of urine at an appropriate time and place ***(Altaf et al., 2010).***

A- Embryology:

The cloaca is divided by the urorectal septum into a dorsal rectum and a ventral urogenital sinus that will give rise to the majority of the urinary bladder. This 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. 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. Before or shortly after birth, the urachus involutes further becoming simply a fibrous cord ***(Stacey et al., 2007).***

B- Anatomy:

The smooth detrusor muscle is in principle a three layered structure, although the differentiation of the individual layers is only clearly visible in the area of the bladder neck. In this section, it is possible to distinguish a longitudinal muscle layer both on the exterior and the interior as well as a circular layer inbetween ***(Altaf et al., 2010).***

The anatomic relations of the bladder are as follows:

1. Anteriorly:

The apex of the bladder points anteriorly towards the superior edge of the pubic symphysis (**Figure 1).** The median umbilical ligament (remnant of the obliterated fetal urachus) anchors the apex of the bladder to the umbilicus and runs up the midline of the abdominal wall in the median umbilical fold of peritoneum. Beneath the apex and adjacent to the inferolateral surfaces of the bladder is the retropubic space of Retzius. This space contains loose fatty tissue and the pubovesical ligaments, which extend from the inferior pubic bones to the bladder neck ***(Altaf et al., 2010).***

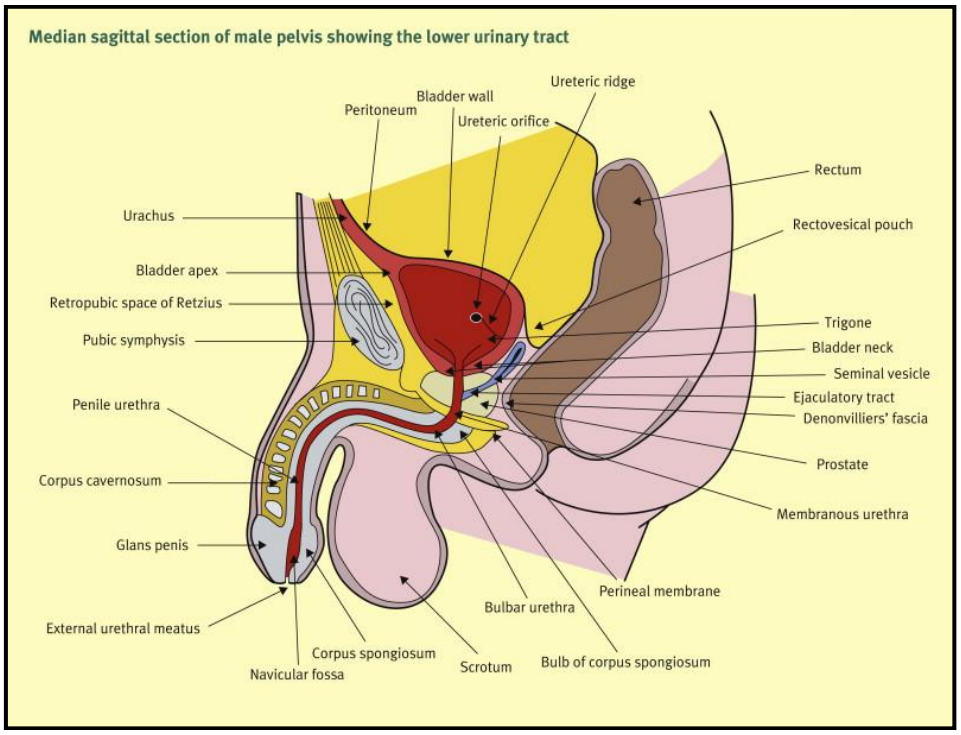

Figure (1): Median sagittal section of male pelvis ***(Altaf et al., 2010).***

2. Superiorly:

The superior surface is covered by peritoneum upon which the sigmoid colon and small intestine usually rest. In females, the anteverted uterus lies against the posterosuperior surface **(Figure 2).** With increasing distension, the bladder rises in the shape of a dome, well above the symphysis pubis, and becomes an abdominal organ which can be palpated and percussed in the suprapubic region. Along the lateral aspect of the superior surface the peritoneum continues to be reflected on to the lateral pelvic walls, leaving a little depression, the paravesical fossa lateral to the bladder on both sides. The

superior surface of the bladder is separated from the base internally by a line joining the two ureters (inter-ureteric line) ***(Altaf et al., 2010).***

3. Inferolaterally:

The inferior surfaces are not covered by peritoneum. The two inferolateral surfaces anteriorly are related to the retropubic pad of fat and the pubic bones. Posteriorly they lie in contact with the obturator internus muscle above and are supported by the levatorani muscle below. The inferolateral surfaces funnel down into the bladder neck ***(Altaf et al., 2010).***

4. Posteriorly:

- **In males:**

The triangular base faces posteriorly towards the rectum. Only the uppermost part of the posterior surface is covered by visceral peritoneum, forming the rectovesical pouch. This line of reflection undergoes little or no change when the bladder is distended. Below this level the ductus deferens and seminal vesicles are adherent to the posterior surface, and the space between the bladder and rectum contains Denonvilliers' rectovesical fascia ***(Altaf et al., 2010).***

- *In females*

The base is united with the anterior vaginal wall and the upper part of the cervix, with loose areolar tissue but no peritoneum intervening **(Figure 2)** ***(Altaf et al., 2010).***

5. Bladder neck and trigone:

The lowest part of the bladder where the inferolateral surfaces meet the base is **the bladder neck**. In the male, the bladder neck lies directly on the prostate, whereas (in the female) the bladder neck and urethra lie in the connective tissue of the anterior vaginal wall. **The triangular trigone** is the lowest part of the bladder channelling into the bladder neck. The trigone is demarcated internally by the two ureteric orifices, inserting obliquely into the bladder posteroinferiorly, and inferiorly by the internal urethral orifice (bladder outlet). The oblique ureteric insertions run intramuscularly and as the bladder contracts, during voiding, act as an important anti-reflux mechanism.

The trigone is histologically and embryologically different from the rest of the bladder and contains a rich plexus of neuronal tissue; it is also the least mobile part of the bladder and is firmly adherent to the underlying muscle ***(Altaf et al., 2010).***

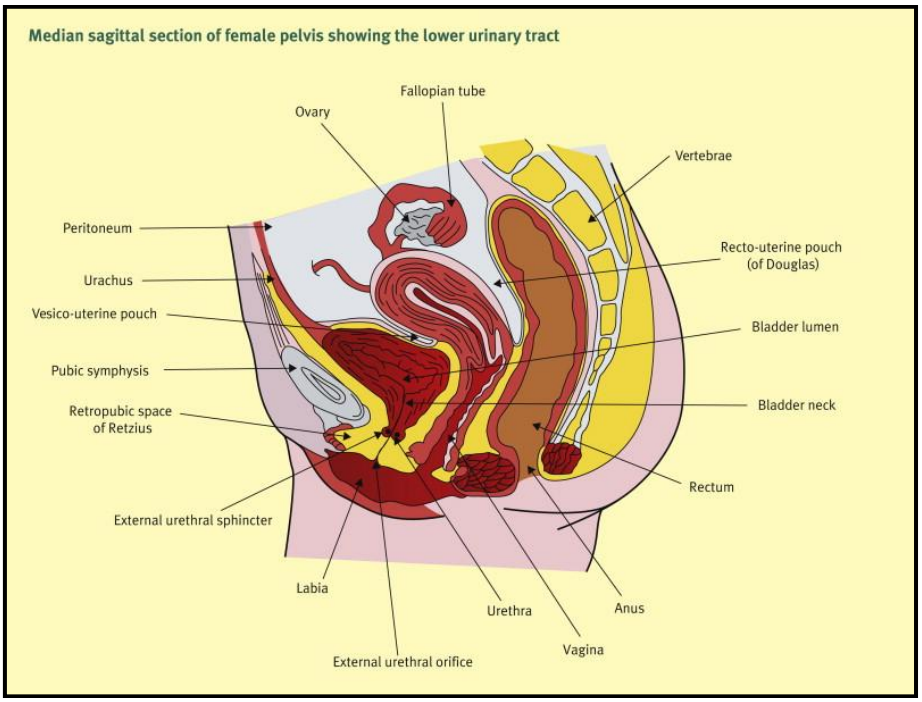

Figure (2): Median sagittal section of female pelvis ***(Altaf et al., 2010).***

C- Histology

The urinary bladder, ureter, and renal pelvis, for the most part, have a similar histologic composition, the innermost layer being an epithelial lining and, extending outward, a lamina propria, smooth muscle (muscularis propria), and adventitia. The superior surface of the bladder comes in contact with parietal peritoneum and hence has a serosa. The anatomic landmarks are used clinically and pathologically to stage patients with urothelial cancer in order to choose therapy and estimate survival. For this reason, it is important to identify them accurately microscopically ***(Stacey et al., 2007).***

1. Urothelium:

The urinary bladder, ureters, and renal pelvis are lined by transitional epithelium. The epithelium of the bladder has been traditionally referred to as ***transitional***, but the term ***urothelium*** is more informative and accurate ***(Rosai et al., 2011).*** 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. 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.

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 on the degree of distension but also on the plane in 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 ***(Stacey et al., 2007).***

Superficial cells

They are in contact with the urinary space. They appear large and elliptical lying as umbrella over the smaller intermediate cells.

They may be binucleated and have abundant eosinophilic cytoplasm. It is possible to see umbrella cells overlying frank carcinoma, so the presence or absence of superficial cells cannot be used as a determining factor of malignancy ***(Stacey et al., 2007).***

The intermediate cell layer:

It may be up to five cells thick in the contracted bladder, but in the distended state, this layer may be inconspicuous or only one cell thick and flattened ***(Stacey et al., 2007).***

The basal layer:

It is composed of cuboidal cells that are evident only in the contracted bladder ***(Stacey et al., 2007).***

Beneath this, lies the suburothelial layer; the urothelium and suburothelium have a high metabolic rate and a rich nervous innervation, and are involved in sensing the fullness of the bladder and modulating bladder function ***(Anand et al., 2008).***

2. Lamina Propria (LP):

The lamina propria lies between the mucosal basement membrane and the muscularis propria (MP). It is composed of dense connective tissue containing a rich vascular network, lymphatic channels, sensory nerve endings, and a few elastic fibers.

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 LP and usually are associated with these vessels ***(Stacey et al., 2007).*** These fascicles of smooth muscles are not connected to the MP; they appear as isolated bundles. Uncommonly, these muscle fibers may present as a continuous layer of muscle within the LP, thus forming a true muscularis mucosae (MM) ***(Stacey et al., 2007).***

These structures can be confused with those of the MP when evaluating the depth of invasion of a bladder neoplasm (particularly in a biopsy specimen), a serious problem since tumor staging and treatment are largely based on the presence or absence of MP invasion. Isolated bundles of muscle immediately adjacent to urothelium, with loose haphazard fiber orientation and irregular outlines, favor MM. Topographic and morphologic variations exist that complicate this evaluation: (1) the more superficial location of the MP and the inconspicuousness of the MM in the trigone; (2) the occurrence of *hyperplastic MM*, defined as MM composed of more than

three layers of muscle fibers appearing as fibers parallel to the surface mucosa or as rounded bundles, this being particularly prominent in the dome ***(Vakar et al., 2010)*** and (3) the presence of superficially located *ureteral* MM at the insertion of the ureter into the bladder ***(Paner et al., 2007).*** **Immunostaining for smoothelin** (a novel smooth muscle-specific marker expressed only in fully differentiated smooth muscle cells) may be of help in the distinction, in the sense that a strong and diffuse reaction is characteristic of MP, in contrast with MM, in which the staining is absent or weak and focal ***(Bovio et al., 2010).***

3. Muscularis Propria (MP):

The MP 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 ***(Stacey et al., 2007).***

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.

Stacey et al. (2007) found that the bladder wall thickness varied mostly with the state of bladder filling and only minimally with age and gender. The bladder wall has a mean thickness of 2.76 mm when empty and 1.55 mm when distended. For staging purposes, the MP 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**.**