Androgen and Estrogen Receptor Expression in Carcinoma Urinary Bladder and Relationship with Clinicopathologic Features

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All India Institute of Medical Sciences, Jodhpur

In partial fulfillment of the requirement for the degree of

Master of Chirurgiae (MCh)

Urology

JUNE 2022 **Dr. NIKITA SHRIVASTAVA**

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DECLARATION

I hereby declare that this thesis titled "Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features" is a bonafide and original research work carried out in partial fulfilment of the requirements for the degree of Master of Chirurgiae (M.Ch.) in Urology under supervision and guidance, in the Department of Urology, All India Institute of Medical Sciences, Jodhpur.

I further state that no part of the thesis has been submitted either in part or in full for any other degree of All India Institute of Medical Sciences or any other institute/university.

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CERTIFICATE

This is to certify that the thesis titled "Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features" is the bonafide work of Dr Nikita Shrivastava carried out under our guidance and supervision, in the Department of Urology, All India Institute of Medical Sciences, Jodhpur.

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Dedicated

to

My Parents

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LIST OF ABBREVIATIONS

AR- androgen receptor

ER alpha- estrogen receptor alpha

ER beta- estrogen receptor beta

GLOBOCAN- Global Cancer Observatory

Ca- Carcinoma

NAT2- N-acetyltransferase 2

GSTM1-Glutathione S-Transferase

HNPCC- Hereditary Non-Polyposis Colon Carcinoma

EBRT- External Beam Radiotherapy

RR- Relative Risk

CT- Computed Tomography

AJCC- American Joint Committee on Cancer

TNM- Tumor, Nodes, and Metastases

WHO- World Health Organization

AUA- American Urological Association

NMIBC- Non Muscle Invasive Bladder Carcinoma

NCCN- National Comprehensive Cancer Network

TURBT- Transurethral Resection of the Bladder Tumor

BCG- Bacillus Calmette Guérin

GC- Gemcitabine plus Cisplatin

MVAC- Methotrexate, Vinblastine, Doxorubicin and Cisplatin

FDA- Food and Drug Administration

MRI- Magnetic Resonance Imaging

CPI- Immune Checkpoint Inhibitors

PD-L1- Programmed Cell Death Ligand-1

FGFR- Fibroblast growth factor receptor

LBD- Ligand Binding Domain

NTD- N-terminal Domain

HSPs- Heat Shock Proteins

DHT- Dihydrotestosterone

AREs- Androgen Response Elements

GnRH- Gonadotropin Releasing Hormone

mRNA- messenger ribonucleic acid

IHC- Immunohistochemistry

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SUMMARY

SUMMARY

STUDY DESIGN: Ambispective Hospital based Cross-sectional Study

OBJECTIVE: To examine expression of androgen receptors and estrogen receptors in patients with urinary bladder carcinoma.

BACKGROUND: Urinary bladder cancer stands eighth amongst causes of cancer related deaths. The advent of immunotherapy for urothelial carcinoma a few years back has been a boon for treatment of metastatic urothelial carcinoma. Unfortunately a small fraction of patients respond to currently available immunotherapeutic agents, and this paves way for assessing androgen and estrogen receptor targeting therapies for effectiveness. For this reason, a precise knowledge of the effect of androgen and estrogen receptors on clinicopathologic characteristics of bladder tumors is needed. Our study is to study the same in the Indian population.

METHODS: A total of 132 patients were included in the study. 116(87.88%) patients were males and 16(12.12%) patients were females. Most of the patients i.e. 42(31.82%) belonged to the age group of 61-70 years. The Histologic stage and grade of 31.82% patients was T1G3 followed by T2 in 29.55%. Only 5 out of 132 patients (3.79%) showed ER alpha positivity, 51.52% of patients had ER beta positivity and AR positivity was seen in 63.64% of patients. Distribution of ER alpha, ER beta and AR positivity was comparable in all age groups and their correlation was not statistically significant. No statistically significant correlation was found between ER alpha positive status and tumor stage and grade in our study.(p value=0.719). The expression of ER beta was more in small sized unifocal tumors.ER alpha expression was found to be higher in metastatic tumors. No statistically significant association was found between pathologic variant, complications, length of hospital stay and ER alpha, ER beta and AR receptor positivity in patients.

CONCLUSION: Our study is the one with the largest sample size conducted on the Indian population, and it revealed results which differ in various aspects from other similar studies of the past. Though AR and ER beta expression is high in bladder tumors and ER alpha expression is positive in a low percentage of patients which is similar to various other studies,

no correlation has been found between their expression and tumor stage and grade.The expression of ER beta was more in small sized unifocal tumors. ER alpha expression was found to be higher in metastatic tumors

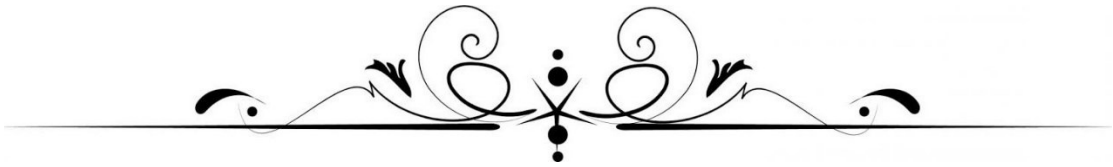

INTRODUCTION

INTRODUCTION

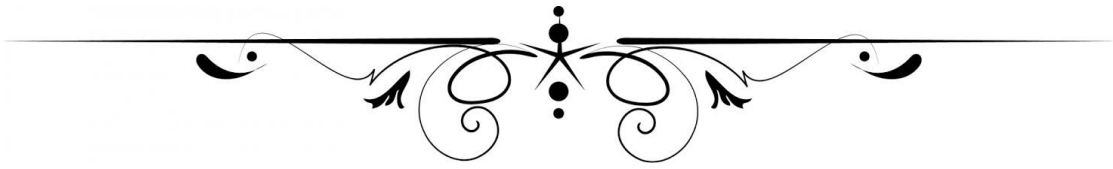
Urinary bladder cancer is the fourth most common cancer in males and eleventh most common in females, out of which nearly 95% have transitional cell carcinoma or urothelial carcinoma histology(1). Globally the male to female ratio is 3.5:1, while in India, it is as alarmingly high as 8.6:1(2). The advent of immunotherapy for urothelial carcinoma a few years back has been a boon for the treatment of metastatic urothelial carcinoma, after a long gap of no approval of new drugs for it for four decades(3). Unfortunately, a small fraction of patients respond to currently available immunotherapeutic agents, and this paves the way for the requirement of more effective targeted therapy.

Emerging preclinical evidence suggests the involvement of sex hormones and their receptor signals in the development and progression of bladder cancer. Meanwhile, previous studies have demonstrated conflicting results on the relationship between the status of sex hormone receptors- including androgen and estrogen receptors in urothelial tumors and histopathological characteristics of the tumors or patient outcomes(3). The role of androgen receptor (AR) signaling in the oncogenesis of prostate cancer is well established, but its role in other human malignancies including bladder cancer is poorly understood. AR receptors have also been proposed to have a role in gender disparity in the presentation of urinary bladder cancer.

Our study is to assess the clinicopathological impact of the expression of androgen receptors (AR) and estrogen receptors(ER) in bladder cancer in a tertiary care center in Rajasthan, India.



REVIEW OF LITERATURE



REVIEW OF LITERATURE

Urinary bladder cancer stands eighth amongst causes of cancer related deaths in males and are 3-4 times more likely to affect males than females (1). But females present with more advanced disease with poorer prognosis (2). The average age of diagnosis is 73, but it can be seen at any age. According to GLOBOCAN 2020, in India, the incidence rate of urinary bladder cancer is 1.6 % and prevalence is 3.57 per 1,00,000 population (3)

Risk factors

- **Smoking/Tobacco**

It is the most well-known etiological agent and increases risk by 2-3 fold(4) Aromatic amines are the primary carcinogens in tobacco smoke that lead to cancer.(4) In those patients who stopped smoking, there was a significant reduction in the risk of CA bladder. This reduction was around 40% within one to four years of cessation and 60% after 25 years.(5)

- **Occupational exposure to chemicals**

It is the second most important risk factor for CA bladder accounting for 20–25% of all cases and is likely to occur in occupations in which dyes, rubbers, textiles, paints, leathers, and chemicals are used (5). The risk due to occupational exposure to carcinogenic aromatic amines is greater after ten years or more of exposure.(5)

- **Genetics**

The most-studied genes associated with bladder cancer are N-acetyltransferase 2 (NAT2) and a deletion of glutathione S-transferase (GSTM1).(4) Both of them metabolize aromatic amines and in case of environmental carcinogen exposure have a significant role. NAT2 detoxifies nitrosamines, regulating the rate of acetylation of aromatic amine compounds found in cigarette smoke. There are several underlying genetic risk factors, which when combined with behavioral, occupational, and environmental exposures, potentiate bladder cancer risk and development.(4)

- **Hereditary**

Hereditary bladder cancer is comparatively rare. The incidence increases 2 fold in relatives of patients with malignancies of bladder. Patients with Lynch syndrome (hereditary non-polyposis colon carcinoma, HNPCC) are at high risk of developing urothelial cancer.

- **Metabolic disorders**

Obesity is a risk factor for bladder cancer.The mechanism by which obesity leads to cancer risk includes insulin resistance, chronic hyperinsulinemia, increased bioavailability of steroid hormones, and localized inflammation. However, the exact mechanism is unknown.(4) Elevated blood pressure and triglycerides are also postulated to be associated with increased risk.(5)

- **Radiotherapy**

Increased rates of secondary bladder malignancies have been reported after external-beam radiotherapy (EBRT) for gynaecological malignancies, with relative risks (RR) of 2-4 (6).

- **Bladder schistosomiasis and chronic urinary tract infection**

Schistosomiasis has been proven to be associated with squamous cell carcinoma of the bladder.(4,5)

Presentation

- Painless gross hematuria - Approximately 80-90% of patients; classic presentation
- Irritative bladder symptoms (eg, dysuria, urgency, frequency of urination) - 20-30% of patients
- Pelvic or bony pain, lower-extremity edema, or flank pain - In patients with advanced disease

Diagnosis

- Upper urinary tract imaging- ultrasonography and CT urography
- Urine studies:

1. Urinalysis with microscopy
2. Urine culture to rule out infection, if suspected
3. Urinary tumor marker testing
4. Urinary cytology

- Bone imaging- only when there is clinical suspicion or symptoms of bone metastases
- Cystoscopy:

The gold-standard test for the diagnosis of bladder cancer is cystoscopy and biopsy. All adult patients who present with gross hematuria and those who are older than 35 years and have microscopic hematuria should undergo cystoscopy.

Staging

The American Joint Committee on Cancer (AJCC) in combination with the International Union Cancer Consortium meets regularly to determine tumor, nodes, and metastases (TNM) staging classification in 2017 which is as follows **(Table 1)**:

T - Primary Tumour	
Tx	Primary tumour cannot be assessed
T0	no evidence of a primary tumor
Ta	Non-invasive papillary carcinoma
Tis	Carcinoma in situ: "flat tumour"
T1	Tumour invades subepithelial connective tissue
T2	Tumour invades muscle T2a Tumour invades superficial muscle (inner half) T2b Tumour invades deep muscle (outer half)
T3	Tumour invades perivesical tissue: T3a microscopically T3b macroscopically (extravesical mass)
T4	Tumour invades any of the following: prostate stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall T4a Tumour invades prostate stroma, seminal vesicles, uterus, or vagina T4b Tumour invades pelvic wall or abdominal wall

N - Regional Lymph Nodes	
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single lymph node in the true pelvis (hypogastric, obturator, external iliac, or presacral)
N2	Metastasis in multiple regional lymph nodes in the true pelvis (hypogastric,

	obturator, external iliac, or presacral)
N3	Metastasis in a common iliac lymph node(s)

M - Distant Metastasis	
M0	No distant metastasis
M1a	Non-regional lymph nodes
M1b	Other distant metastasis

Stage 0a	Ta N0 M0
Stage 0is	Tis N0 M0
Stage I	T1 N0 M0
Stage II	T2a N0 M0 T2b N0 M0
Stage III	T3a N0 M0 T3b N0 M0 T4a N0 M0
Stage IV	T4b N0 M0 Any T N1-3 M0 Any T Any N M1

Pathology.

Bladder cancer is broadly divided into three clinical types - non-muscle invasive, muscle-invasive, and metastatic. All three of them vary in clinical phenotype, biological behaviour, prognosis and management. Approximately 70% to 80% of bladder tumors are non–muscle invasive at presentation with 60% to 70% as Ta, 20% to 30% as T1, and approximately 10% as carcinoma in situ(4). In addition to these, bladder cancer demonstrates several variants of different histologies that bear significant impact on the prognosis and management of patients.(10)

Broadly dividing, Urothelial carcinoma comprises about 90% of cases, squamous cell carcinoma 3-7% and adenocarcinoma 0.5-2%. Normal urothelium comprises multilayered

mucosa, 4 to 7 cells thick, which features the transition between pseudostratified columnar and nonkeratinizing squamous epithelium. Cells mature from the basal basement membrane cells, which are small and cuboidal, to intermediate cells to superficial umbrella cells in an orderly manner. The surface is capped with large umbrella cells that are in the form of asymmetrical entities. These umbrella cells form a barrier between urine and bladder and prevent toxins from affecting urothelial cells. Malignant transformation is a continuum from cells turning hyperplastic, then progress to atypia, dysplasia and finally malignancy.(4)

Based on the 2004/2016 World Health Organization (WHO) classification, currently the urothelial malignancies are classified into following subtypes (11,12)(**Table 2**)

Urothelial carcinoma	Infiltrating urothelial carcinoma Urothelial carcinoma with divergent differentiation With squamous differentiation With glandular differentiation With trophoblastic differentiation Nested urothelial carcinoma (including large nested variant) Microcystic urothelial carcinoma Micropapillary urothelial carcinoma Lymphoepithelioma-like urothelial carcinoma Plasmacytoid / signet ring / diffuse urothelial carcinoma Sarcomatoid urothelial carcinoma Giant cell urothelial carcinoma Poorly differentiated urothelial carcinoma Lipid rich urothelial carcinoma Clear cell (glycogen rich) urothelial carcinoma Noninvasive urothelial lesions Urothelial carcinoma in situ

	Noninvasive papillary urothelial carcinoma, low grade Noninvasive papillary urothelial carcinoma, high grade Papillary urothelial neoplasm of low malignant potential Urothelial papilloma Inverted urothelial papilloma Urothelial proliferation of uncertain malignant potential Urothelial dysplasia
Squamous cell neoplasms	Pure squamous cell carcinoma Verrucous carcinoma Squamous cell papilloma
Glandular neoplasms	Adenocarcinoma, NOS Enteric Mucinous Mixed Villous adenoma
Urachal carcinoma	
Tumors of Müllerian type	Clear cell carcinoma Endometrioid carcinoma
Neuroendocrine tumors	Small cell neuroendocrine carcinoma Large cell neuroendocrine carcinoma Well differentiated neuroendocrine tumor Paraganglioma
Melanocytic tumors	Malignant melanoma

	Nevus Melanosis
Mesenchymal tumors	Rhabdomyosarcoma Leiomyosarcoma Angiosarcoma Inflammatory myofibroblastic tumor Perivascular epithelioid cell tumor Benign Malignant Solitary fibrous tumor Leiomyoma Hemangioma Granular cell tumor Neurofibroma
Urothelial tract hematopoietic and lymphoid tumors	
Miscellaneous tumors	Carcinoma of Skene, Cowper and Littre glands Metastatic tumors and tumors extending from other organs Epithelial tumors of the upper urinary tract Tumors arising in a bladder diverticulum Urothelial tumors of the urethra

Treatment

Non–muscle-invasive bladder carcinoma

- Table 3:AUA risk stratification for NMIBC(9)

Low risk	Intermediate risk	High risk
Papillary urothelial neoplasm of low malignant potential Low grade urothelial carcinoma Ta <=3cm solitary	low grade urothelial carcinoma T1 or >3cm or Multifocal or Recurrence within 1 year High grade urothelial carcinoma Ta and <=3cm and solitary	High grade urothelial carcinoma CIS or T1 or >3cm or Multifocal Very high risk features(any): BCG unresponsive Variant histologies Lymphovascular invasion Prostatic urethra invasion

- National Comprehensive Cancer Network (NCCN) recommendations for NMIBC are as follows (9):
- Standard treatment for non–muscle invasive bladder cancer (NMIBC) is a complete transurethral resection of the bladder tumor (TURBT)
- a restage TURBT is recommended 2-6 weeks after the initial resection in any of the following situations (4,5):

1) After incomplete initial TURBT
2) If there is no muscle in the specimen after initial resection, with exception of Ta low-grade tumors
3) In all T1 tumors
4) In all high-grade tumors.

- Intravesical chemotherapy is generally used as prophylactic or adjuvant therapy after complete endoscopic resection. The agent of choice to be decided on the basis of AUA risk stratification:

Low risk- surveillance

Intermediate risk- intravesical mitomycin or gemcitabine(preferred) or surveillance

- One postoperative intravesical dose (within 24 h, but usually immediately after resection) has been shown to decrease recurrence, but not progression, in patients of low-risk NMIBC
- Immediate intravesical chemotherapy is avoided when TURBT was extensive or perforation is suspected or the tumor appears invasive or high grade
- Immediate intravesical chemotherapy can be succeeded by a induction of intravesical chemotherapy for 6 weeks(once per week)
- **Table 4**:recommendations for treatment of high-grade NMIBC:

Intravesical bacillus Calmette-Guérin (BCG) naïve-	Very high risk features-	radical cystectomy(preferred) or BCG
	No very high risk features-	BCG(preferred) or radical cystectomy
BCG unresponsive or intolerant		radical cystectomy(preferred) or valrubicin or pembrolizumab

Muscle-invasive bladder cancer (MIBC)

- NCCN recommendations for treatment of muscle-invasive disease are as follows (9) :
- TURBT is the initial diagnostic procedure after CT/MRI imaging of the abdomen, pelvis, chest, to help identify the clinical stage of bladder cancer.
- Radical cystectomy is the primary treatment for all muscle-invasive disease, with strong consideration for cisplatin-based neoadjuvant chemotherapy (category 1 recommendation),
- Bladder preservation following TURBT with concurrent chemotherapy and radiation is an alternative therapy for patients with multiple medical comorbidities or who refuse radical cystectomy

Metastatic bladder cancer

In spite of initial therapeutic measures, about 50% of patients with muscle invasive bladder cancer may develop recurrence or metastases(30). The first line of therapy in suitable patients of metastatic bladder cancer continues to be platinum-based combination chemotherapy with gemcitabine plus cisplatin (GC) or methotrexate, vinblastine, doxorubicin and cisplatin (MVAC) (31,32). The advent of immune checkpoint inhibitors has significantly boosted the treatment of metastatic bladder cancer after a long gap of about 40 years. Food and Drug Administration (FDA) has approved five immune checkpoint inhibitors(CPI), namely programmed cell death ligand-1 (PD-L1) inhibitors (atezolizumab, avelumab, and durvalumab) and anti-PD-1 antibodies (pembrolizumab and nivolumab) for management of advanced and metastatic bladder malignancies .(33-38) Atezolizumab and pembrolizumab have been approved as first-line therapy in cisplatin-ineligible patients of metastatic bladder cancer with high expression of PD-L1.(39) Unfortunately, poor efficacy limits the use of CPIs, with significant response in just 20% of patients. In the present scenario there are very limited treatment options for bladder cancer patients who show no response or show progression on CPIs. Enfortumab vedotin, a novel antibody drug conjugate, has shown promising results in bladder cancer patients not responding to prior chemotherapy and immune checkpoint inhibitors(40). Fibroblast growth factor receptor (FGFR) inhibitor erdafitinib is another promising agent which acts on patients with genetic alterations of FGFR3 or FGFR2. It also shows good results in patients who showed progression on prior platinum based chemotherapy (41). But only around 10% metastatic bladder cancer patients harbor the susceptible genetic alterations, limiting its use. Giving the poor five-year survival rate of metastatic bladder cancer patients, there is a great need of development of novel targeted therapies in bladder cancer(16)

Targeted therapies for metastatic bladder cancer- the need of hour

Though the current histopathologic classification for bladder cancer has improved its management, dealing with progression and recurrence on existing therapeutic measures still remains in question. Adding to it, accurate prediction of response to specific therapies cannot be made. Bladder tumors show vast heterogeneity with respect to both clinical behavior and histopathology.(13)Many theories about tumorigenesis are suggestive of involvement of ditomonous genetic pathways in urinary bladder carcinoma. Patients with low grade malignancy have a better disease- specific survival, although they may have recurrence,

occasionally with progression in grade. In contrast, invasive high grade malignancies generally show poor prognosis, even when treated aggressively. Other than aggressiveness of tumor, gender disparity in incidence and nature has also been related to genetics. The sex steroid pathways have been postulated to play a predominant role in it.(21)

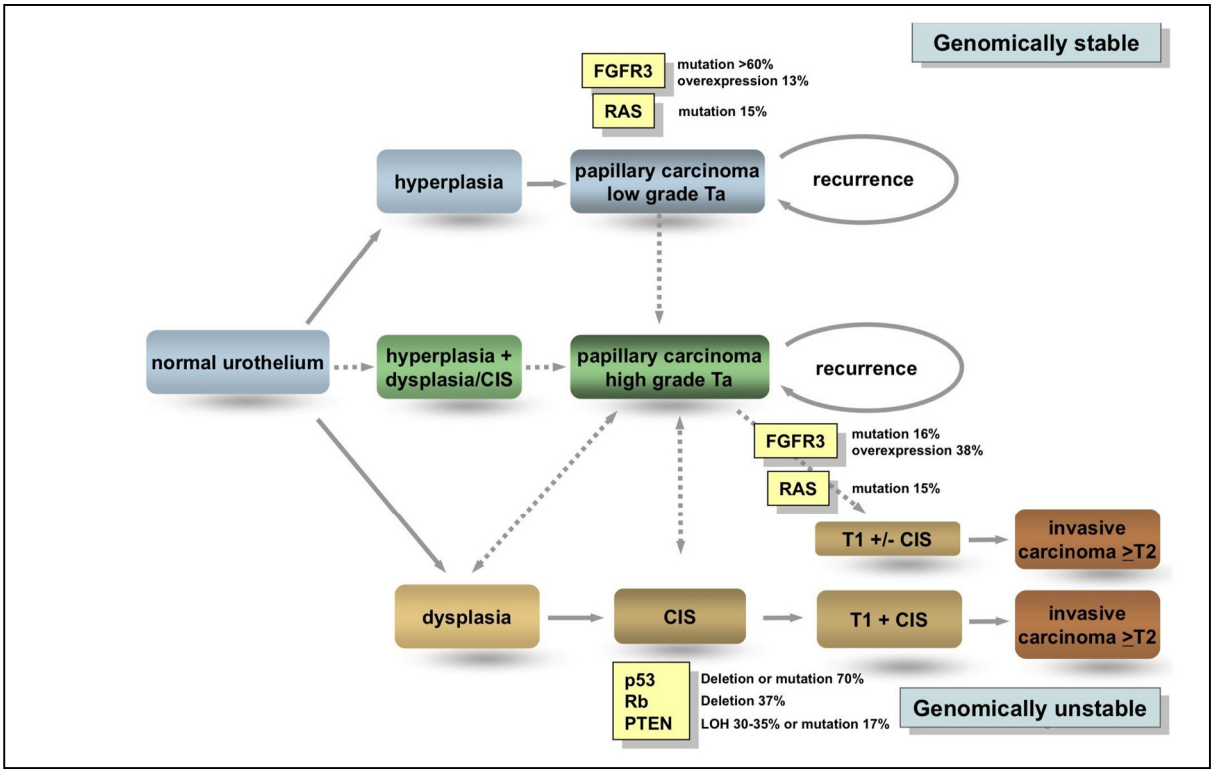


Figure 1:potential pathways during urothelial tumorigenesis(13)

Androgen receptors

Structure and function of androgen receptors(AR)

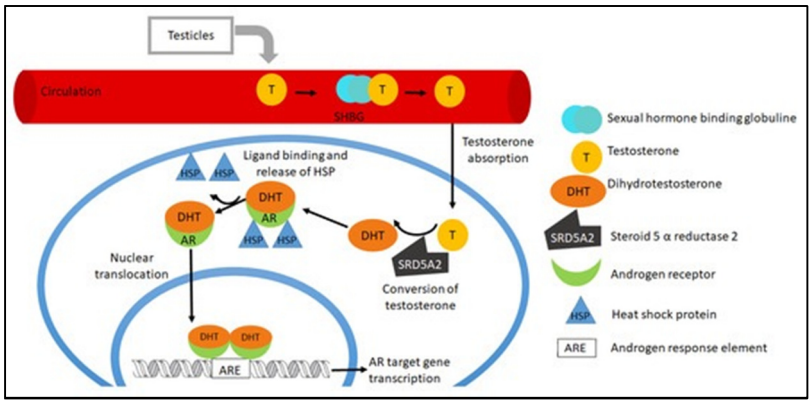


Figure 2:Genomic androgen receptor pathway.(42)

The androgen receptor is a member of the steroid hormone receptor family. It comprises 3 domains- a deoxyribonucleic acid (DNA) binding, ligand binding (LBD) and an N-terminal domain (NTD) (16). When its ligands are absent, the AR is bound to heat shock proteins (HSPs) in the cytoplasm. On binding to its ligands like dihydrotestosterone (DHT), it is translocated from cytoplasm to the nucleus, where it regulates the transcription of its target genes, which are also known as androgen response elements (AREs). In prostate cancer the role of AR in oncogenesis is well recognized, where the cornerstone of therapy is formed by AR targeting agents such as gonadotropin releasing hormone (GnRH) agonist/antagonists and AR antagonists such as enzalutamide(16). Other than prostate cancer, AR has been found to play a role in pathogenesis of other cancers also like triple-negative breast cancer and bladder cancer(16).

Physiologic functions of AR in bladder

The prostate, membranous urethra and urinary bladder are derived from the endoderm of urogenital sinus. AR expression in normal urothelium, smooth muscles, submucosa and neurons has been described in primate and human bladder, though urinary bladder is not traditionally considered to be an androgen responsive organ. (44). The role of AR in normal development of bladder remains incompletely understood. In preclinical models, androgen deprivation has been found to be associated with a significant decrease in total and smooth muscle mass of bladder, decrease in function of autonomic nerves and reduced capacity of bladder. Moreover these effects were reversible with testosterone supplementation (45). These studies show that there is a significant role of AR signaling in normal embryonic development and functioning of the urinary bladder.

Role of androgen receptors in bladder cancer

Traditionally bladder cancer has not been seen as an androgen driven malignancy. But there could be some link between bladder malignancy and androgens, as suggested by strong male preponderance of ca bladder (16). One of the reasons for the gender disparity has been explained by higher exposure to cigarette smoking and occupational exposure. But men continue to have three to four times increased risk of developing bladder cancer than women even when accounting for addictions, lifestyle and environmental factors. Women are generally found to present with more advanced malignancy and with poorer prognosis (16). This predilection can be explained by the differences in AR signaling in both the genders.

Miyamoto et al. (43) studied mice models and found that the oncogenic effects of carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN) was dependent on AR signaling. The incidence of urothelial cancer was higher in AR wild type male mice treated with BBN in comparison to female mice in this study(92% vs. 42%, respectively). On the contrary, none of the AR knockout mice developed malignancy(43).

Androgen receptor expression in bladder cancer and correlation with clinicopathologic features

Expression of sex steroidal hormone by immunohistochemistry (IHC) is seen in tumor tissue in 11% to 55% of patients with bladder carcinoma.(14) The studies on AR expression in bladder carcinoma have shown contrasting results. Some studies have shown an increase in AR expression in tumor tissue compared to normal urothelial tissue (20), whereas others have reported its down regulation in cancerous cells(17-19). A meta-analysis comprising nine studies found AR expression to be similar in cancerous tissue in comparison to normal urothelium. The expression was also similar in muscle-invasive and non-muscle invasive tumors (21). Surprisingly, in comparison to low grade tumors, AR expression was found to be lower in high-grade tumors. On the other hand, Elzamy et al. (22) found that expression of AR was seen in 35% of patients with urothelial carcinoma and is related to high grade tumors and muscle invasiveness. Other Studies on relation of AR expression in urothelial carcinoma to prognosis have shown discordance in results. According to Nam et al. expression of AR is related to reduced risk of recurrence in patients with non muscle invasive carcinoma (23). On the contrary, no significant association was found between AR expression and outcomes in other similar studies (22). A recent study by Sikic et al. correlated AR mRNA expression in their institutional cohort of 41 patients of ca bladder and 323 patients of TCGA dataset (25). Although no correlation was found between gender and AR expression, a significantly worse disease-free survival and overall survival was found in females in whom expression of AR mRNA above median level was found, as compared to men in whom this was not observed. So their conclusion was that AR mRNA expression can be used in women as an independent prognostic marker for disease-free survival. Also, they analysed that expression of AR mRNA was higher in non muscle invasive than in muscle-invasive carcinoma (23). A study by Yasui et al. correlated recurrence of non muscle invasive disease and AR expression in Japanese population (26). It was a retrospective study where AR expression was correlated with recurrence-free survival in 53 patients with non muscle invasive disease. Although no

significant difference in recurrence-free survival was found between the groups expressing high AR and low AR, multivariate analysis showed tumor size <3 cm and female gender to be independent predictors of shorter recurrence-free survival (24). Such discrepancy in results could be due to differences in the assays used for assessing AR expression. It has also been postulated that the expression of AR might change during bladder cancer progression and AR expression might not be a true reflection of AR signaling(22). On the basis of this hypothesis, Bergerot et al. (14) assessed AR expression by IHC and quantitative polymerase chain reaction and gave an AR activity score which would reflect expression of AR responsive genes. Out of the 37 patients with muscle invasive cancer included in this study, AR expression was noticed in 54% of patients but no correlation was found with AR activity score(22).There is a single Indian study by Pachauri et al over the role of AR in carcinoma of urinary bladder which evaluated 20 cases of urothelial carcinoma and found that expression of AR signifies bad prognosis and targeting AR and androgen may provide novel chemopreventive and therapeutic approaches for the management of bladder cancer(28).

Study	sample size	method of assessment	key findings
Birtle et al., 2004	17	IHC	2þ/3þ AR expression in 52% of samples No expression in normal urothelium
Boorjian et al., 2004	49	IHC	53% of samples expressed AR Prevalence of AR expression decreased in: Tumor tissue (53%) vs. normal urothelium (86%) MIBC (21%) vs. NMIBC (75%) High grade (49%) vs. low grade (89%)

Ide et al., 2017	2049	meta-analysis	Similar AR expression in normal tissue vs. tumor tissue AR expression strongly correlated with: Gender (male vs. female) Tumor grade (low grade vs. high grade)
Kauffman et al., 2011	72	IHC	Decreased AR expression in: Tumor vs. normal tissue (51% vs. 84%) MIBC vs. NMIBC
Kashiwagi et al., 2016	99	IHC	Decreased AR expression in tumor (20%) vs. normal tissue (57%) No correlation with DSM
Elzamy et al., 2018	106	IHC	AR positivity in 35% AR expression more frequent in MIBC (41%) vs. NMIBC (19%) High grade (52%) vs. low grade (15%)

			No correlation of AR expression with RFS
Nam et al., 2014	169	IHC	AR expression seen in 37% of samples AR expression associated with improved RFS/PFS in NMIBC
Sikic et al., 2019	41(institutional cohort)	gene expression analysis	Significantly lower AR mRNA expression in MIBC vs. NMIBC Similar AR mRNA expression in males vs. females
	323(The Cancer Genome Atlas (TCGA) cohort)	gene expression analysis	AR mRNA expression highest in luminal subtype AR expression associated with worse DFS and OS

Table 5:Summary of prior studies investigating the prevalence and significance of AR expression in urothelial carcinoma(16).

Targeting androgen receptors in bladder cancer- preliminary results

There have been few preclinical studies on the use of androgen deprivation therapy in management of bladder cancer(46,47,48). There is some preclinical evidence that shows resistance to cisplatin-based chemotherapy mediated by AR. The antitumor efficacy of combined treatment enzalutamide and cisplatin in ca bladder was studied by Tyagi et al. He found that these agents together synergistically inhibit tumor cell growth which is due to

increment in pro-apoptotic signaling and reduction in mesenchymal markers(49) . Huang et al. also reported similar results in a study of androgen receptor (AR)-degradation enhancer ASC-J9 in muscle-invasive bladder cancer (miBCa) cells. He found that ASC-J9 and cisplatin, when given together, cause increase in expression of the cell cycle inhibitor p21 gene and pro-apoptotic BAX gene, and reduction in anti-apoptotic gene BCL-2. Due to this reason better results in tumor suppression were obtained than with cisplatin alone(50).

Though preclinical evidence has provided rationale for targeting AR with or without cisplatin in bladder cancer, clinical data which proves efficacy of AR inhibitors in bladder cancer is lacking. A phase 1 trial was carried out which studied the safety and efficacy of enzalutamide, cisplatin and gemcitabine together in patients of metastatic ca bladder(NCT02300610) (51). Patients of advanced ca bladder who had not received any previous treatment were enrolled to evaluate two doses of enzalutamide (80 mg and 160 mg daily) with six cycles of standard doses of cisplatin and gemcitabine. In total, 10 patients were enrolled including six in the dose escalation phase and four in the dose expansion part of the study. The maximum tolerated dose of enzalutamide was found to be 160 mg daily and no dose limiting toxicities were noted. The combination showed good efficacy with complete response in one female patient who had strongly positive AR expression, and partial response and stable disease in four and two patients respectively. This was the first study to demonstrate safety and efficacy of enzalutamide with cisplatin and gemcitabine in metastatic bladder cancer, and it's testing in larger trials is needed(51).

The variability in results from the above trials call for the need of systematic clinical studies to investigate the role of androgen receptors in bladder cancer. Also,the prognostic and predictive significance of AR in various stages of bladder carcinoma needs to be studied.(16)

Estrogen receptors

The physiological functions of estrogen compounds in the body are modulated primarily by the estrogen receptor subtypes alpha (ERα) and beta (ERβ). These proteins mainly act on the cell nucleus, regulating transcription of specific target genes by binding to associated DNA regulatory sequences. In humans, both receptor subtypes are expressed in many cells and tissues, and they control key physiological functions in various organ systems. ERα is predominant in mammary gland, uterus, ovary (thecal cells), bone, male reproductive organs (testes and epididymis), prostate (stroma), liver, and adipose tissue. ERβ is found mainly in

the prostate (epithelium), bladder, ovary (granulosa cells), colon, adipose tissue, and immune system.(60)

Role of estrogen receptors in bladder cancer

In certain tissues such as breast, estrogens and estrogen metabolites have been proven to act as carcinogens. Estrogen receptors have been found to induce DNA damage in various tissues.(52) In contrast, protective role of estrogens against bladder cancer initiation in N--butyl--N-[4--hydroxybutyl]--nitrosamine (BBN)-induced bladder cancer in rodent models have been shown by Okajima et al.and Tanahashi et al.(53,54). Studies showed greater risk of bladder cancer in postmenopausal women than premeno-pausal women(55). This was supported by the finding that women who attain menopause at a younger age have increased risk of bladder cancer.(56) The results of studies involving immunohistochemical staining of ERα and ERβ in different stages and grades of bladder cancer have been inconsistent. Bolenz et al. worked on ERα immunohisto-chemical staining and found that ERα expres-sion was associated with lower tumour stage and ERα expression in bladder cancer patients who had lymph node metastases was absent. He concluded that ERα prevents lymph node metastasis in bladder cancer .(57) Croft et al. also showed similar results in metastatic bladder cancer and found all metastatic foci are negative of ERα expression in bladder cancer patients(58). Miyamoto et al. also found a lower expression of ERα in high grade tumours (23%) and tumours invading the muscularis propria (19%) than in low grade tumours (51%).(59) On the other hand, they found higher expression of ERβ in high grade tumours (58%) and muscle invasive tumours (67%). Still there has not been a consistent agreement on the role of ERα in bladder cancer cell growth. Different studies show different results on the role of ERα in promoting or inhibiting cancer cell growth in the bladder. These discrepancies could be due to heterogeneous nature of tumours and differences in study design(52).The above mentioned data can facilitate development of new therapeutic agents that selectively enhance ERα expression, suppress ERβ, or act upon the downstream target genes of these receptors.(52)

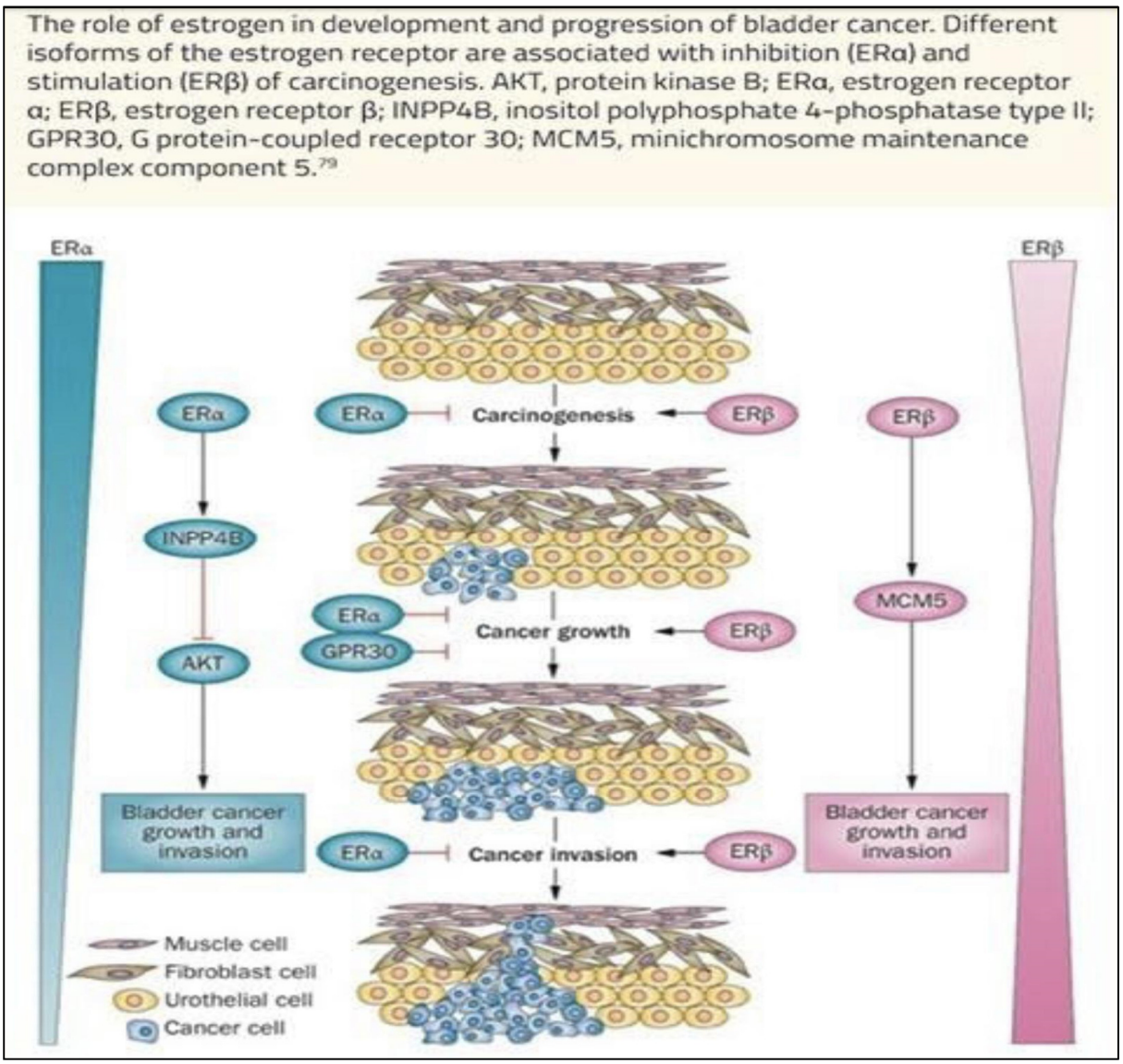


Figure 3: the role of estrogen in development and expression of bladder cancer (52)

To conclude, an inverse relationship has been found between expression of AR and advanced disease, which can also be explained by the biphasic nature of behavior of AR during the natural course of the bladder cancer. ERα expression has been found to be protective against the development of bladder cancer, whereas expression of ERβ is greater in high-grade and muscle-invasive bladder cancer. In a country like India, more studies are needed where the etiology, biology, behavior, and treatment of bladder cancer varies vastly across the community(14). Our intention is to further study the role of AR and ER in carcinoma of urinary bladder in the Indian population presenting in our institute.

AIM & OBJECTIVES

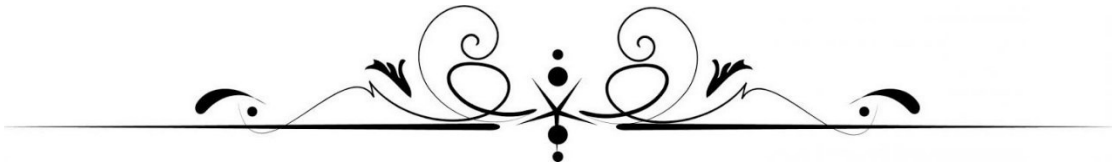
AIM AND OBJECTIVES

Primary objective:

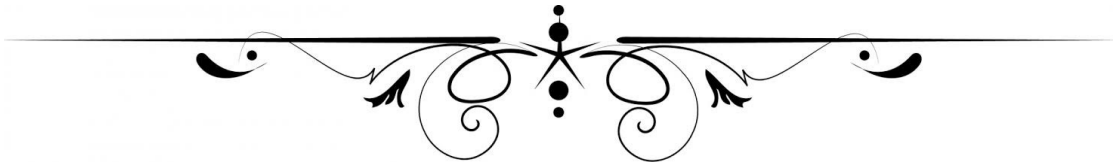
To examine the expression of androgen receptors and estrogen receptors in patients with Ca urinary bladder.

Secondary objective:

1. To study the association of expression of AR and ER receptors with pathological stage and grade of tumor.
2. To establish their expression in different types of bladder carcinoma.
3. To study the correlation of various clinical parameters with androgen and estrogen receptor expression.



MATERIALS & METHODS



MATERIALS AND METHODS

PLACE OF STUDY: Department of Urology and Department of pathology, All India Institute of Medical Science, Jodhpur, Rajasthan, India.

TIME PERIOD- October 2020 to January 2022

STUDY DESIGN: Ambispective Hospital based Cross-sectional Study

PATIENT SELECTION:

INCLUSION CRITERIA:

Prospective cases: All patients diagnosed with urinary bladder mass and undergoing surgery for the same, either transurethral resection of bladder tumor (TURBT), TUR biopsy or radical cystectomy and diagnosed with urinary bladder neoplasm on histopathological examination (HPE)

Retrospective cases: archived paraffin embedded blocks of diagnosed cases of urinary bladder neoplasm on histopathological examination (HPE) from a time period of January 2019 to October 2020 were recruited.

EXCLUSION CRITERIA:

1. Non-neoplastic pathology on HPE
2. Patients not willing to participate in the study
3. Inadequate sampling

SAMPLE SIZE: The following equation was used for sample size calculation

$$N = \frac{\sigma_1 + \sigma_2\ (Z_{1-\alpha} + Z_{1-\beta})^2}{(M_1 + M_2)^2}$$

Where σ1 and σ2 are standard deviations in group 1 and group 2, α is alpha error, β is beta error, M1 and M2 are mean from group 1 and 2 respectively, and Z is a constant.

Boorjian S et al 2004 found that 75% of superficial tumors expressed AR. Using this for sample size calculation and using the above formula, we estimated that **136** bladder cancer patients will be needed at 95% CI, 10% relative precision and 5% contingency.

METHODOLOGY

A clearance from the Ethical Committee of Institution was obtained prior to the investigation (Ethical clearance number AIIMS/IEC/2019-20/963).Samples were taken in an ambispective manner. For both prospective and retrospective cases, baseline assessment of the patients including the demographic characterization, medical history, physical examination and radiological findings as per ultrasound abdomen and pelvis, computed tomography (CT scan) or magnetic Resonance Imaging (MRI) was noted. Type of surgery performed and perioperative parameters were noted such as complications (if any), degree of haematuria post-surgery and length of hospital stay were noted. For retrospective cases this data was procured from medical record files and online database of AIIMS, Jodhpur. The previous diagnosed cases of urinary bladder neoplasms in the Department of Pathology from January 2019 to December 2021 were re-assessed and the corresponding blocks were taken out and submitted for IHC testing.

Histopathologic assessment methodology for all the samples:

Haematoxylin and eosin stained slides of prospective and retrospective cases were analyzed for the histologic type, grade and stage of the tumor, according to current WHO classification. For IHC testing, blocks were selected based on the adequate tumor tissue and were subjected to immunohistochemical staining for ER-alpha, ER-beta and AR.

Immunohistochemical Technique

The antibodies, used in this study, along with their clones and concentration are listed Table below:

Table 6:- Antibodies used in the study with their clones and concentration

IHC stain	Dilution	Manifacturing company	Clone	Buffer
ERα	1:100	Thermoscientific	SP1	Citrate
AR	1:100	Thermoscientific	Polyclonal	Citrate
ERβ	1:50	Medaysis	ERb455	Tris-EDTA

Appropriate positive controls were used in each IHC testing. Breast tissue was taken as control for ERα, prostate for AR and testis for ERβ. For negative control, primary antibodies were omitted.

Procedure for Immunohistochemistry

Sections of 4μ thickness were cut from paraffin blocks and mounted on poly-l-lysine coated slides. They were dried overnight at 37° C. The slides were dewaxed in xylene and rehydrated in a series of graded alcohol. This was followed by 3 washes in Phosphate Buffer Saline (PBS) for 5 minutes each. Endogenous peroxidase activity was blocked by freshly prepared 0.3 % hydrogen peroxide in methanol for 20 minutes, followed by 3 washes in PBS. Antigen retrieval was done by pressure method Citrate buffer (pH 6.0) for ERα and AR while Tris-EDTA buffer for ERβ. After cooling slides were removed from the instrument and dipped in distilled water (30 seconds) and transferred to PBS. This was followed by washing in PBS 3 times of 5 minutes each at room temperature. The primary antibody with appropriate dilution (Table abov e) was then applied to the sections and kept at room temperature for one and half hour to 2 hours in a moist chamber.This was followed by three washes with PBS for 5 minutes each and incubated with biotinylated secondary antibody for 30 minutes. After 3 washes with PBS for 5 minutes each the sections was covered with freshly prepared Di-amino benzidene , DAB solution for 3 minutes and rinsed with water. Counterstaining was done with haematoxylin for 5 minutes followed by one wash with Scotts tap water. Then the sections were dehydrated, washed with xylene and mounted with Dextrene Pthylate Xylene [DPX]. Appropriate control slides were used with each testing.

Evaluation

For each IHC stains, cases showing nuclear expression in more than 10% of tumor cells were considered positive for these immunohistochemical markers.

The patients included in the study were analyzed for distribution of ER alpha, ER beta and AR in their tumor tissues. Also, the expression of these three receptors was correlated separately with following parameters:

- Age
- Gender
- Histologic stage and grade
- Pathologic variant
- Size of tumor
- Multifocality of tumor
- Metastases at presentation
- Complications
- Length of hospital stay post surgery
- Association of ER alpha, ER beta and AR with each other

STATISTICAL ANALYSIS

The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means ± SD and as median with 25th and 75th percentiles (interquartile range). The data normality was checked by using Kolmogorov-Smirnov test. The cases in which the data was not normal, we used non parametric tests. The following statistical tests were applied for the results:

1. The association of the variables which were quantitative and not normally distributed in nature were analysed using Mann-Whitney Test and Independent t test was used for association of normally distributed data between two groups.
2. The association of the variables which were qualitative in nature were analysed using Chi-Square test. If any cell had an expected value of less than 5 then Fisher's exact test was used.

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 21.0.

For statistical significance, p value of less than 0.05 was considered statistically significant.

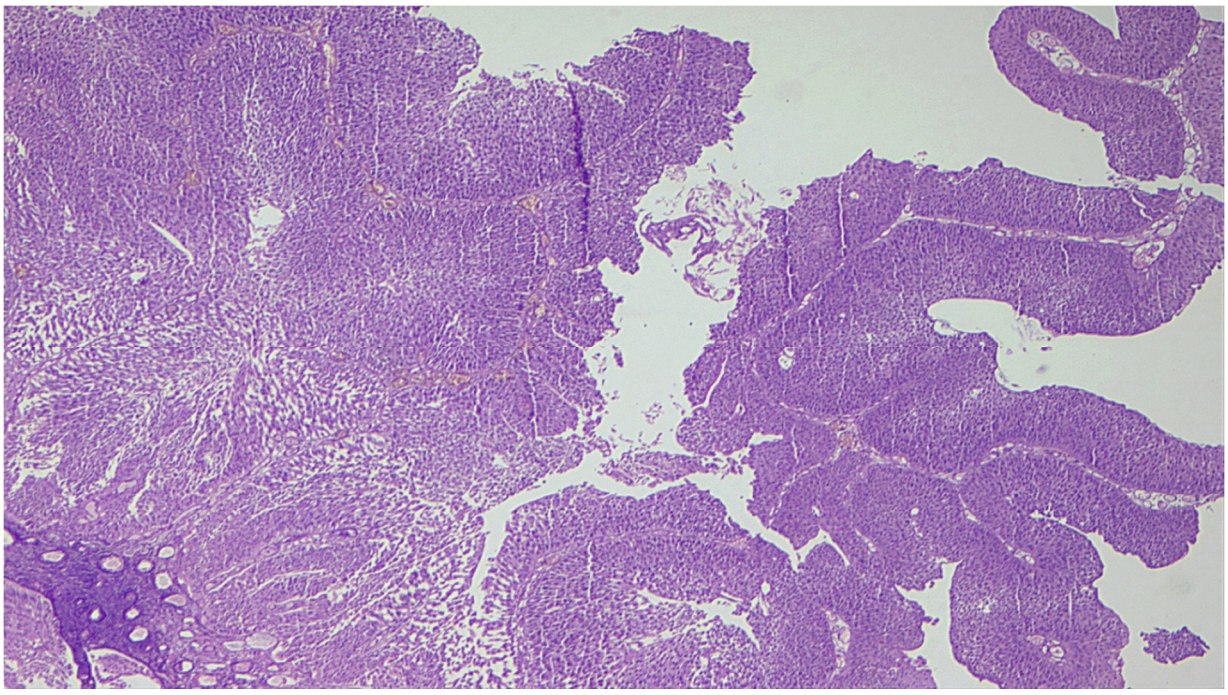


Fig 4: Low power microphotograph of low grade papillary urothelial carcinoma. [Hematoxylin &eosin, 100x]

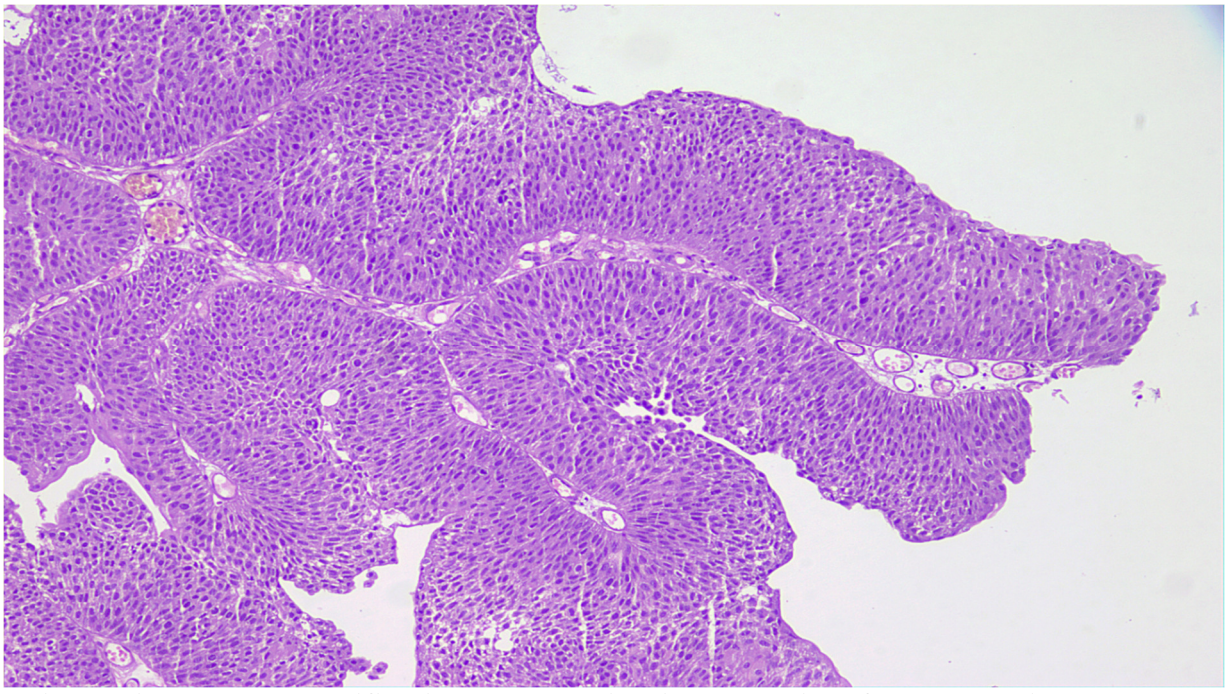


Fig 5: High power magnification shows maintained polarity of the urothelial cells and presence of umbrella cell layer on the surface. [Hematoxylin &eosin, 200x]

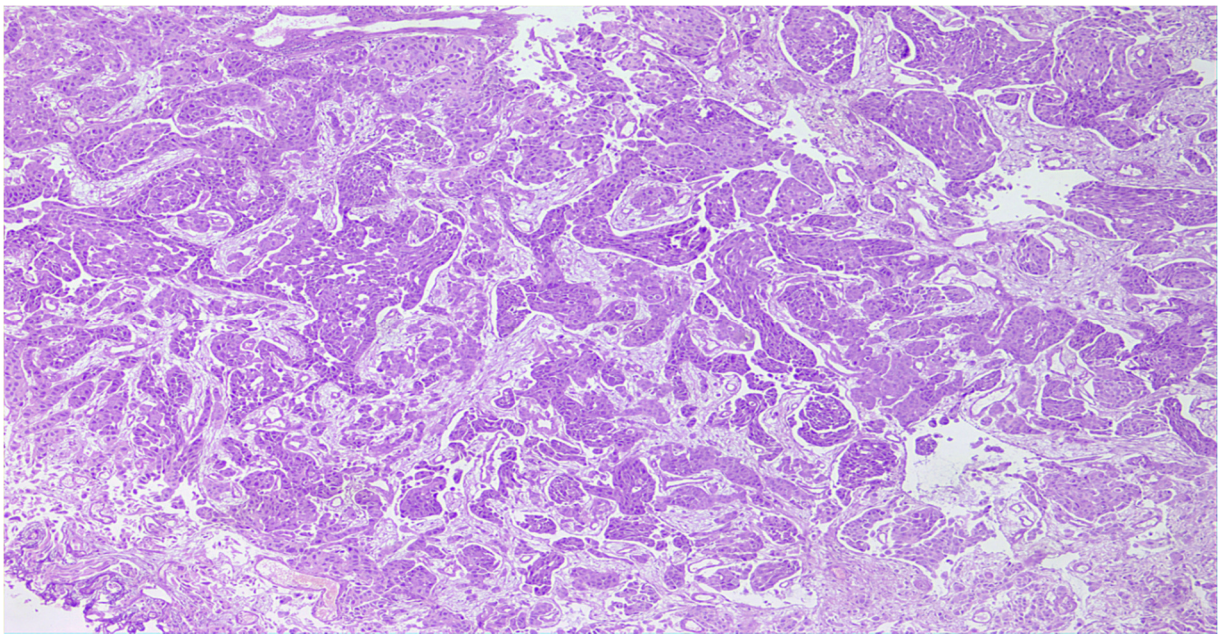


Fig 6: High grade urothelial carcinoma arranged in irregular islands and nests. Also note lamina propria invasion. [Hematoxylin &eosin, 100x]

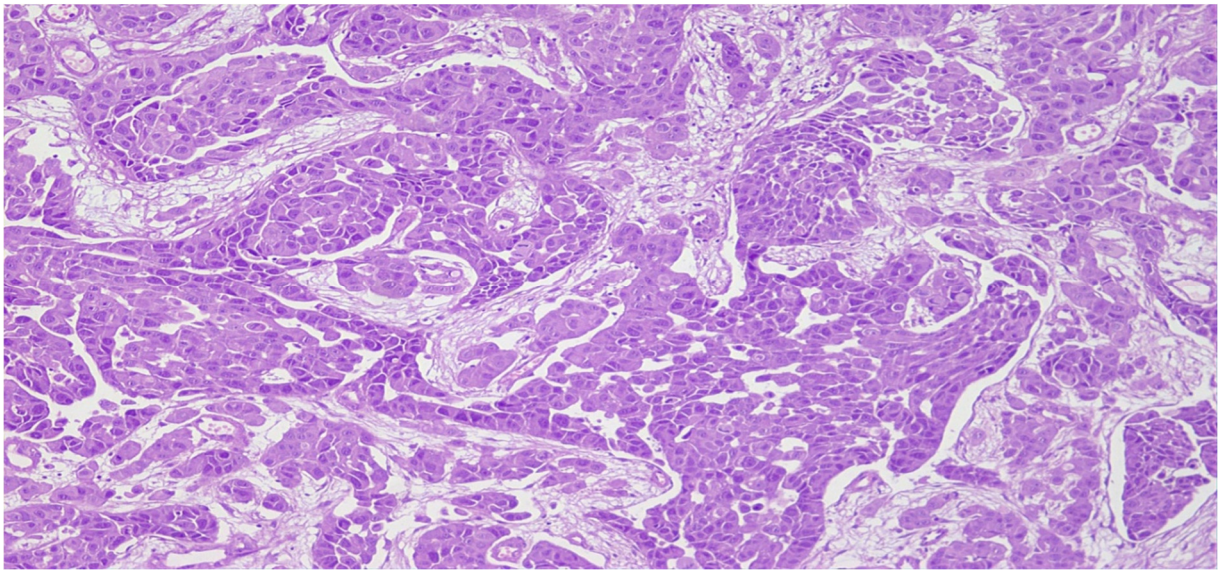


Fig 7: Moderate to marked nuclear atypia of tumor cells with frequent mitotic figures [Hematoxylin &eosin, 200x]

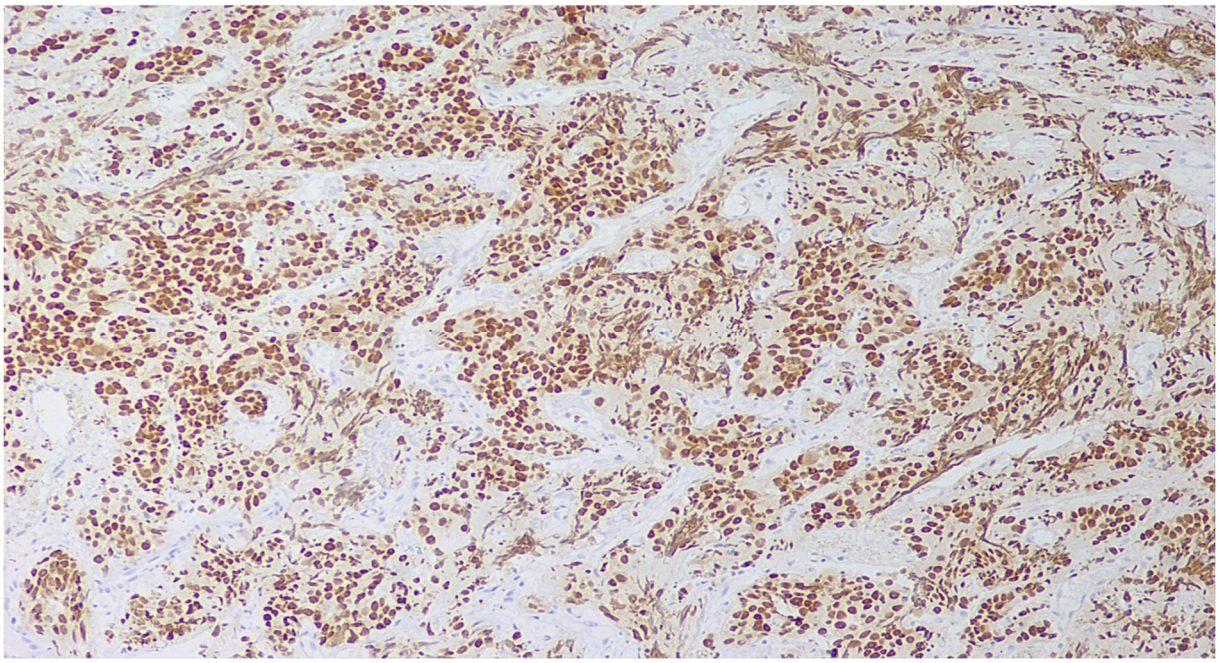


Fig 8: Strong nuclear expression for Androgen receptor in tumor cells [Immunohistochemistry, 200X]

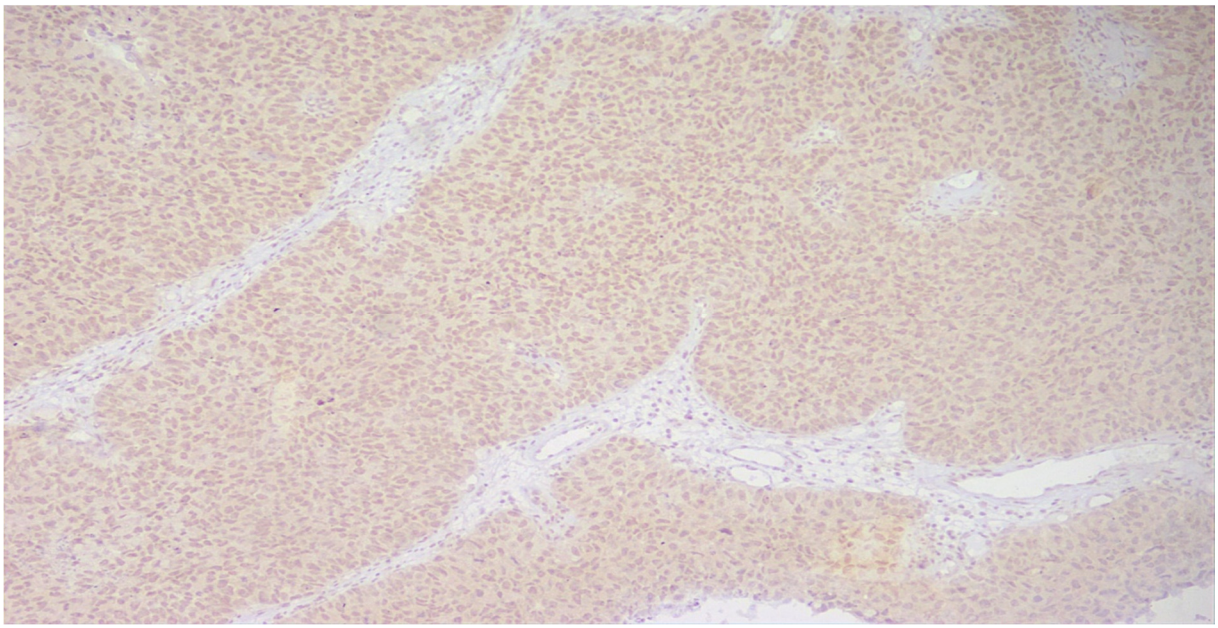


Fig 9: Moderate to strong Nuclear expression for Estrogen receptor beta in tumor cells [Immunohistochemistry, 200X]

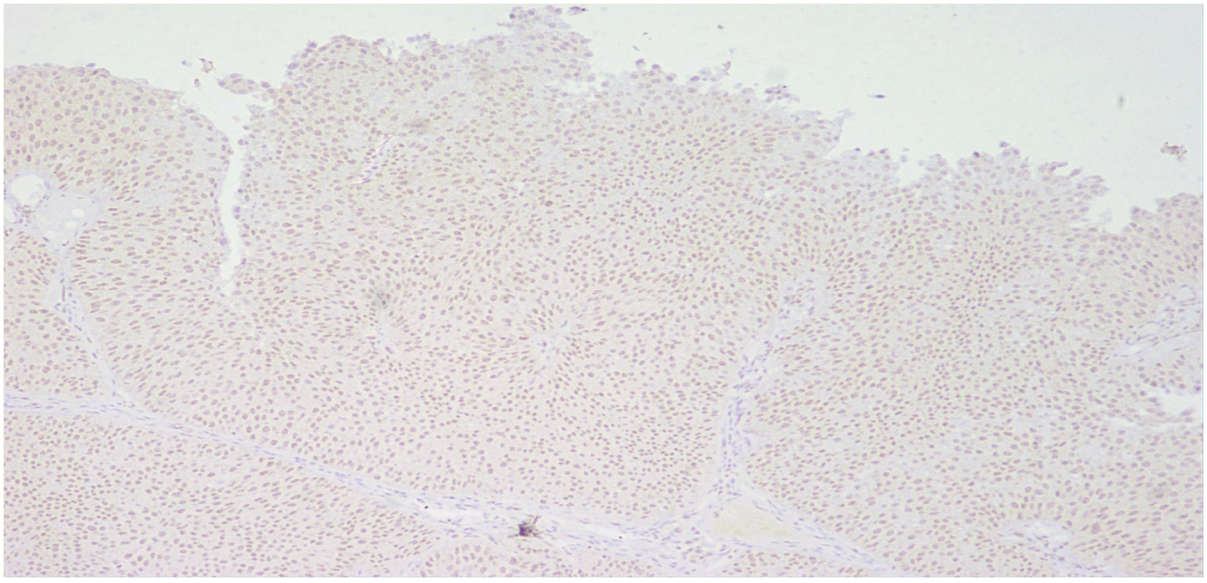
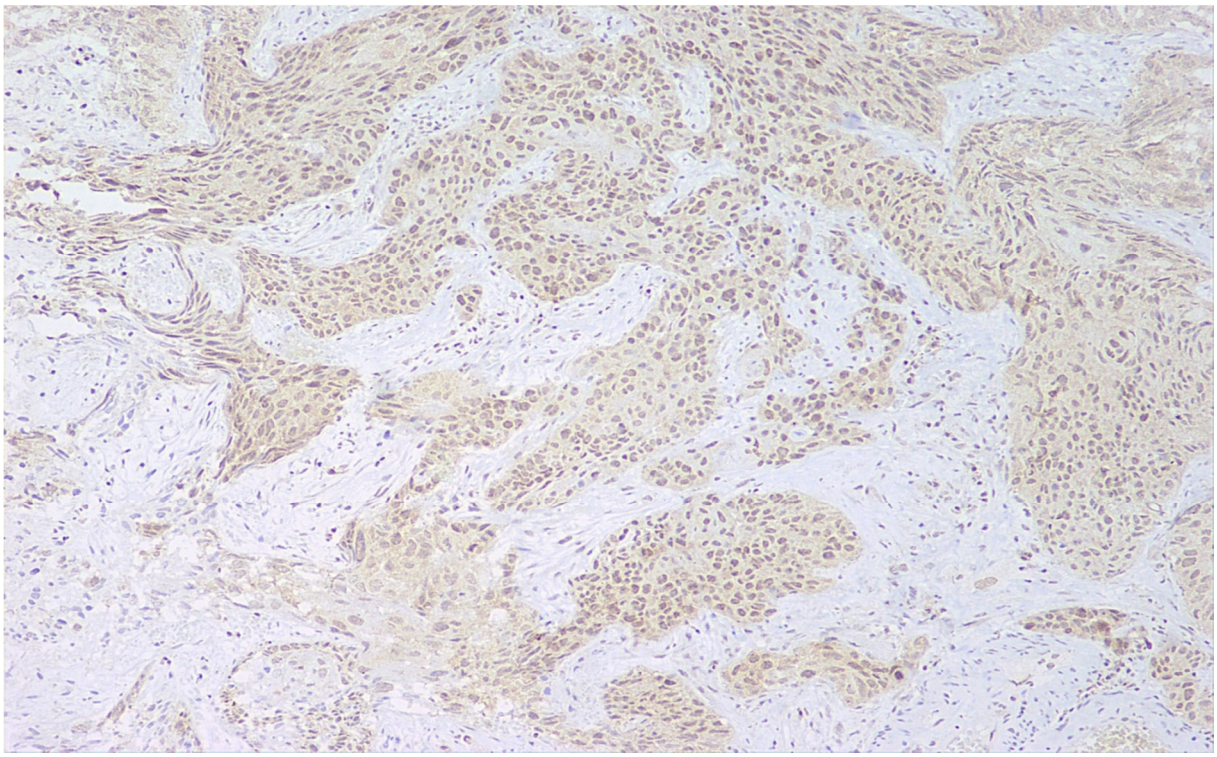
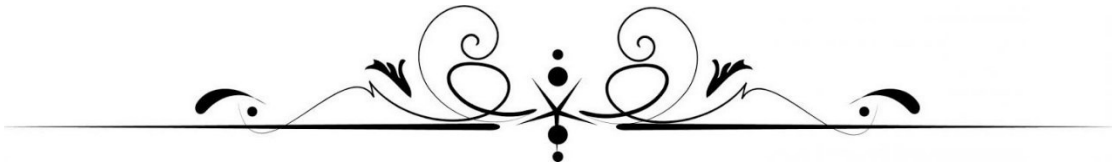


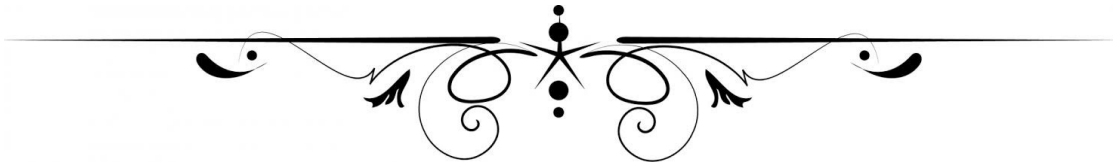
Fig 10 A. Strong nuclear expression for ER alpha in low-grade papillary urothelial carcinoma



10 B. Strong nuclear expression for ER alpha in high-grade urothelial carcinoma.



OBSERVATIONS & RESULTS



OBSERVATIONS AND RESULTS

The study was conducted in the Department of Urology and Department of Pathology, All India Institute of Medical Science, Jodhpur, Rajasthan, India from October 2020 to January 2022 in an ambispective manner. A total of 132 patients taken in both prospective and retrospective manner, which were diagnosed with urinary bladder mass and undergone surgery for the same, either transurethral resection of bladder tumor (TURBT), TUR biopsy, random bladder biopsies or radical/palliative cystectomy and diagnosed with urinary bladder neoplasm on histopathological examination (HPE) were included in the study. For both prospective and retrospective cases, baseline assessment of the patients were done, type of surgery underwent by the patient and perioperative parameters were noted and results are as follows.

Table 7:-Distribution of age (years) of patients.

Age(years)	**Frequency**	**Percentage**
31-40	7	5.30%
41-50	19	14.39%
51-60	38	28.79%
61-70	42	31.82%
71-80	22	16.67%
>80	4	3.03%
Mean ± SD	61.28 ± 12.1	
Median(25th-75th percentile)	61.5(54-69.25)	
Range	31-101	

Most of the patients i.e. 42(31.82%) belonged to the age group of 61-70 years followed by 51-60 years (28.79%), 71-80 years (16.67%) and 41-50 years(14.39%). Comparatively lesser number of patients were there at extremes of age, with 5.30% patients of age <40 years and 3.03% patients of age>80 years. Mean value of age(years) of patients was 61.28 ± 12.1 with median(25th-75th percentile) of 61.5(54-69.25). It is shown in table 6.

Table 8:-Distribution of gender of patients.

Gender	Frequency	Percentage
Female	16	12.12%
Male	116	87.88%
Total	132	100.00%

116(87.88%) patients were males and 16(12.12%) patients were females.

It is shown in table 7.

Table 9:-Distribution of histologic stage and grade of tumors.

Histologic stage and grade	Frequency	Percentage
TaG1	27	20.45%
TaG3	4	3.03%
T1G1	14	10.61%
T1G3	42	31.82%
T2	39	29.55%
T3b	5	3.79%
T4a	1	0.76%
Total	132	100.00%

The Histologic stage and grade of maximum patients (31.82%) was high grade with invasion to lamina propria (T1G3). About 20.45% of patients had low grade non invasive carcinoma(TaG1), 3.03% patients had non invasive high grade carcinoma (TaG3), 10.61% patients had low grade carcinoma with invasion to lamina propria(T1G1), 29.55% of patients had muscle invasive disease (T2), 5 patients had T3b disease and 1 out of 132 patients had T4a disease.It is shown in table 8

Table 10:-Distribution of pathological variants of tumors in patients.

Pathological variant	Frequency	Percentage
Urothelial	108	81.82%
Chondrosarcomatous	1	0.76%
Plasmacytoid	1	0.76%
Sarcomatoid	1	0.76%
Small round blue cell tumor	1	0.76%
Squamous	16	12.12%
Adenocarcinoma	4	3.03%
Total	132	100.00%

108 patients had pure urothelial variant followed by squamous differentiation in 16 patients, adenocarcinoma in 4 patients, chondrosarcomatous differentiation in 1, plasmacytoid variant in 1, sarcomatoid variant in 1 and small round blue cell tumor in 1 out of 132 patients. It is shown in table 9.

Table 11:-Distribution of size (in cm) of tumors.

Size(cm)	Frequency	Percentage
<3 cm	36	27.27%
3 to 5 cm	51	38.64%
>5 cm	45	34.09%
Mean ± SD	4.29 ± 2.32	
Median(25th-75th percentile)	4(2.65-5.6)	
Range	0.5-16	

In the majority of patients (38.64%), size of bladder tumor was between 3 to 5 cm followed by >5 cm in 34.09% of patients. Size was <3 cm in only 36 out of 132 patients (27.27%). Mean value of size of patients was 4.29 ± 2.32cm with median(25th-75th percentile) of 4(2.65-5.6).

Table 12:-Distribution of focality of bladder malignancy in patients.

Focality	Frequency	Percentage
Unifocal	83	62.88%
In bladder diverticulum	1	0.76%
Multifocal	48	36.36%
Total	132	100.00%

Majority of bladder tumors were unifocal (62.88%). 36.36% of patients had multifocal tumors. In 1 of the patients tumor was found in bladder diverticulum. It is shown in table 11.

Table 13:-Distribution of metastasis at presentation of patients.

Metastasis at presentation	Frequency	Percentage
No	126	95.45%
Yes	6	4.55%
Total	132	100.00%

Distant metastasis at presentation was present in only 6 out of 132 patients (4.55%).

Table 14:-Distribution of surgery done on patients.

Surgery done	Frequency	Percentage
TURBT	91	68.94%
Palliative cystectomy	2	1.52%
Random bladder biopsies	1	0.76%
RC with IC	19	14.39%
RC with neobladder	1	0.76%
Restage TURBT	1	0.76%
TUR biopsy	17	12.88%
Total	132	100.00%

Majority of patients (68.94%) in our study underwent TURBT. Radical cystectomy with ileal conduit (RC with IC) was done in 19 patients, radical cystectomy (RC) with neobladder in 1 patient, TUR biopsy in 19 patients, palliative cystectomy in 2 patients, random bladder biopsies in 1 patient, and restage TURBT in 1 out of 132 patients.It is shown in table 13.

Table 15:-Distribution of complications in patients undergoing TURBT/TUR Biopsy.

Complications	Frequency	Percentage(of total cases)
None	101	76.5%
AKI	1	0.76%
Cardiac arrest	1	0.76%
Hematuria	5	3.79%
Iatrogenic urinary bladder perforation	4	3.03%
Total	112	84.84%

Table 16:-Distribution of complications in patients undergoing radical/palliative cystectomy

Complications	Frequency	Percentage(of total cases)
None	11	8.33%
Abdominal ileus	1	0.76%
Anemia	1	0.76%
Hypotension	1	0.76%
Intestinal obstruction	1	0.76%
Left lower limb DVT post op	1	0.76%
Fever	2	1.52%
Wound dehiscence	1	0.76%
Respiratory complications	1	0.76%
Total	20	15.15%

Table 17:-Distribution of complications in patients undergoing TURBT/TUR Biopsy as per Clavein Dindo classification.

Clavein dindo classification	Frequency	Percentage(of total cases)
None	101	76.51%
1	0	0%
2	0	0%
3a	0	0%
3b	9	6.84%
4a	1	0.76%
4b	0	0%
5	1	0.76%
Total	112	84.84%

Table 18:-Distribution of complications in patients undergoing radical/palliative cystectomy as per Clavein Dindo classification.

Clavein dindo classification	Frequency	Percentage(of total cases)
None	11	8.33%
1	3	2.27%
2	3	2.27%
3a	0	0%
3b	1	0.76%
4a	1	0.76%
4b	1	0.76%
5	0	0%
Total	20	15.15%

Most of the patients (84.85%) had an uneventful course in hospital postoperatively. The various complications seen in patients undergoing TURBT/TUR biopsy are listed in table 14 and in patients undergoing radical/palliative cystectomy are listed in table 15. As per Clavein Dindo classification, as shown in table 16 for transurethral surgeries, 9 out of 112 patients had grade 3b complications i.e. requiring intervention under anaesthesia, 1 patient had acute kidney injury and 1 patient died immediate postoperatively due to cardiac arrest. Similarly table 17 shows complication in patients undergoing cystectomies as per Clavein Dindo

classification. 6 of 20 patients had grade 1 and 2 complications. 1 patient had intestinal obstruction requiring surgical intervention(grade 3b), 1 patient had grade 4a and 1 had grade 4b (Multi organ failure) complications.

Table 19:-Distribution of length of hospital stay post-surgery of patients.

Length of hospital stay post-surgery	**Frequency**	**Percentage**
<3 days	75	56.82%
3 to 7 days	35	26.52%
>7 days	18	13.64%
Death	4	3.03%
Mean ± SD	3.7 ± 3.91	
Median(25th-75th percentile)	2(2-4)	
Range	1-30	

In 56.82% of patients, postoperative length of hospital stay was <3 days; 26.52% of patients stayed for 3 to 7 days and 13.64% had postoperative hospital stay of >7 days . 4 out of 132 patients died postoperatively. Mean value of length of hospital stay of patients was 3.7 ± 3.91 with median(25th-75th percentile) of 2(2-4). It is shown in table 18.

Table 20:-Distribution of ER alpha in tumor cells in patients.

ER alpha	**Frequency**	**Percentage**
Negative	127	96.21%
Positive	5	3.79%
Total	132	100.00%

ER alpha was negative in most of the patients. Only 5 out of 132 patients (3.79%) showed ER alpha positivity.

Table 21:-Distribution of ER beta in tumor cells of patients.

ER beta	**Frequency**	**Percentage**
Negative	64	48.48%
Positive	68	51.52%
Total	132	100.00%

In 51.52% of patients, ER beta was positive.

Table 22:-Distribution of AR in tumor cells of patients.

AR	Frequency	Percentage
Negative	48	36.36%
Positive	84	63.64%
Total	132	100.00%

AR positivity was seen in 63.64% of patients.

Table 23:-Association of age (years) of patients with ER alpha.

Age(years)	Negative(n=127)	Positive(n=5)	Total	P value
31-40	7 (100%)	0 (0%)	7 (100%)	0.752‡
41-50	19 (100%)	0 (0%)	19 (100%)	
51-60	35 (92.11%)	3 (7.89%)	38 (100%)	
61-70	41 (97.62%)	1 (2.38%)	42 (100%)	
71-80	21 (95.45%)	1 (4.55%)	22 (100%)	
>80	4 (100%)	0 (0%)	4 (100%)	
Mean ± SD	61.31 ± 12.23	60.6 ± 8.65	61.28 ± 12.09	0.899*
Median(25th-75th percentile)	62 (54-69.5)	55 (55-69)	61.5 (54-69.25)	
Range	31-101	53-71	31-101	

*** Independent t test, ‡ Fisher's exact test**

Table 24:-Association of age(years) with ER beta.

Age(years)	Negative(n=64)	Positive(n=68)	Total	P value
31-40	2 (28.57%)	5 (71.43%)	7 (100%)	0.057‡
41-50	13 (68.42%)	6 (31.58%)	19 (100%)	
51-60	22 (57.89%)	16 (42.11%)	38 (100%)	
61-70	14 (33.33%)	28 (66.67%)	42 (100%)	
71-80	12 (54.55%)	10 (45.45%)	22 (100%)	
>80	1 (25%)	3 (75%)	4 (100%)	
Mean ± SD	60.53 ± 12.03	61.99 ± 12.2	61.28 ± 12.09	0.492*
Median(25th-75th percentile)	60 (51.75-69.25)	64.5 (55.75-69.25)	61.5 (54-69.25)	
Range	38-101	31-90	31-101	

*** Independent t test, ‡ Fisher's exact test**

Table 25:-Association of age(years) with AR.

Age(years)	Negative(n=48)	Positive(n=84)	Total	P value
31-40	1 (14.29%)	6 (85.71%)	7 (100%)	0.328‡
41-50	10 (52.63%)	9 (47.37%)	19 (100%)	
51-60	14 (36.84%)	24 (63.16%)	38 (100%)	
61-70	16 (38.10%)	26 (61.90%)	42 (100%)	
71-80	5 (22.73%)	17 (77.27%)	22 (100%)	
>80	2 (50%)	2 (50%)	4 (100%)	
Mean ± SD	61 ± 11.86	61.44 ± 12.29	61.28 ± 12.09	0.841*
Median(25th-75th percentile)	60 (53.5-69)	62 (54.75-70)	61.5 (54-69.25)	
Range	31-90	32-101	31-101	

*** Independent t test, ‡ Fisher's exact test**

Distribution of ER alpha, ER beta and AR positivity was comparable in age groups and was not statistically significant (table 22,23,24)

Table 26:-Association of gender with ER alpha.

Gender	Negative(n=127)	Positive(n=5)	Total	P value
Female	14 (87.50%)	2 (12.50%)	16 (100%)	0.111‡
Male	113 (97.41%)	3 (2.59%)	116 (100%)	
Total	127 (96.21%)	5 (3.79%)	132 (100%)	

‡ Fisher's exact test

Table 27:-Association of gender with ER beta.

Gender	Negative(n=64)	Positive(n=68)	Total	P value
Female	6 (37.50%)	10 (62.50%)	16 (100%)	0.348[§]
Male	58 (50%)	58 (50%)	116 (100%)	
Total	64 (48.48%)	68 (51.52%)	132 (100%)	

[§] **Chi square test**

Table 28:-Association of gender with AR.

Gender	Negative(n=48)	Positive(n=84)	Total	P value
Female	6 (37.50%)	10 (62.50%)	16 (100%)	0.92[§]
Male	42 (36.21%)	74 (63.79%)	116 (100%)	
Total	48 (36.36%)	84 (63.64%)	132 (100%)	

[§] **Chi square test**

ER alpha, ER beta and AR positivity was comparable in all age groups.

Table 29:-Association of histologic stage and grade with ER alpha.

Histologic stage and grade	Negative(n=127)	Positive(n=5)	Total	P value
T1G1	13 (92.86%)	1 (7.14%)	14 (100%)	0.719‡
TaG1	27 (100%)	0 (0%)	27 (100%)	
T1G3	40 (95.24%)	2 (4.76%)	42 (100%)	
TaG3	4 (100%)	0 (0%)	4 (100%)	
T2	37 (94.87%)	2 (5.13%)	39 (100%)	
T3b	5 (100%)	0 (0%)	5 (100%)	
T4a	1 (100%)	0 (0%)	1 (100%)	
Total	127 (96.21%)	5 (3.79%)	132 (100%)	

‡ **Fisher's exact test**

No statistically significant correlation was found between ER alpha positive status and tumor stage and grade.(p value=0.719).

Table 30:-Association of histologic stage and grade with ER beta.

Histologic stage and grade	Negative(n=64)	Positive(n=68)	Total	P value
T1G1	5 (35.71%)	9 (64.29%)	14 (100%)	0.382‡
TaG1	10 (37.04%)	17 (62.96%)	27 (100%)	
T1G3	22 (52.38%)	20 (47.62%)	42 (100%)	
TaG3	1 (25%)	3 (75%)	4 (100%)	
T2	23 (58.97%)	16 (41.03%)	39 (100%)	
T3b	2 (40%)	3 (60%)	5 (100%)	
T4a	1 (100%)	0 (0%)	1 (100%)	
Total	64 (48.48%)	68 (51.52%)	132 (100%)	

‡ **Fisher's exact test**

Distribution of ER beta positivity was comparable in various histologic stages and grades and no statistically significant difference was found. (T1G1(64.29%) vs TaG1(62.96%) vs T1G3(47.62%) vs TaG3(75%) vs T2(41.03%) vs T3b(60%) vs T4a(0%)). (p value=0.382). It is shown in table 29.

Table 31:-Association of histologic stage and grade with AR.

Histologic stage and grade	Negative(n=48)	Positive(n=84)	Total	P value
T1G1	5 (35.71%)	9 (64.29%)	14 (100%)	0.214‡
TaG1	7 (25.93%)	20 (74.07%)	27 (100%)	
T1G3	13 (30.95%)	29 (69.05%)	42 (100%)	
TaG3	1 (25%)	3 (75%)	4 (100%)	
T2	18 (46.15%)	21 (53.85%)	39 (100%)	
T3b	4 (80%)	1 (20%)	5 (100%)	
T4a	0 (0%)	1 (100%)	1 (100%)	
Total	48 (36.36%)	84 (63.64%)	132 (100%)	

‡ **Fisher's exact test**

No statistically significant correlation was found between AR positive status and tumor stage and grade.(p value=0.214).

Table 32:-Association of pathological type with ER alpha.

Pathological type	Negative(n=127)	Positive(n=5)	Total	P value
Adenocarcinoma	4 (100%)	0 (0%)	4 (100%)	1‡
Chondrosarcomatous	1 (100%)	0 (0%)	1 (100%)	
Plasmacytoid	1 (100%)	0 (0%)	1 (100%)	
Sarcomatoid	1 (100%)	0 (0%)	1 (100%)	
Small round blue cell tumor	1 (100%)	0 (0%)	1 (100%)	
Squamous	16 (100%)	0 (0%)	16 (100%)	
Urothelial	103 (95.37%)	5 (4.63%)	108 (100%)	
Total	127 (96.21%)	5 (3.79%)	132 (100%)	

‡ **Fisher's exact test**

Table 33:-Association of pathological type with ER beta.

Pathological type	Negative(n=64)	Positive(n=68)	Total	P value
Adenocarcinoma	3 (75%)	1 (25%)	4 (100%)	0.584‡
Chondrosarcomatous	1 (100%)	0 (0%)	1 (100%)	
Plasmacytoid	0 (0%)	1 (100%)	1 (100%)	
Sarcomatoid	1 (100%)	0 (0%)	1 (100%)	
Small round blue cell tumor	0 (0%)	1 (100%)	1 (100%)	
Squamous	8 (50%)	8 (50%)	16 (100%)	
Urothelial	51 (47.22%)	57 (52.78%)	108 (100%)	
Total	64 (48.48%)	68 (51.52%)	132 (100%)	

‡ **Fisher's exact test**

Table 34:-Association of pathological type with AR.

Pathological type	Negative(n=48)	Positive(n=84)	Total	P value
Adenocarcinoma	3 (75%)	1 (25%)	4 (100%)	0.384‡
Chondrosarcomatous	0 (0%)	1 (100%)	1 (100%)	
Plasmacytoid	0 (0%)	1 (100%)	1 (100%)	
Sarcomatoid	0 (0%)	1 (100%)	1 (100%)	
Small round blue cell tumor	0 (0%)	1 (100%)	1 (100%)	
Squamous	8 (50%)	8 (50%)	16 (100%)	
Urothelial	37 (34.26%)	71 (65.74%)	108 (100%)	
Total	48 (36.36%)	84 (63.64%)	132 (100%)	

‡ **Fisher's exact test**

No statistically significant correlation was found between ER alpha, ER beta and AR positivity and pathologic variants of bladder tumors.

Table 35:-Association of size(cm) with ER alpha.

Size(cm)	Negative(n=127)	Positive(n=5)	Total	P value
<3 cm	35 (97.22%)	1 (2.78%)	36 (100%)	0.736‡
3 to 5 cm	48 (94.12%)	3 (5.88%)	51 (100%)	
>5 cm	44 (97.78%)	1 (2.22%)	45 (100%)	
Mean ± SD	4.31 ± 2.34	3.74 ± 1.55	4.29 ± 2.32	0.729†
Median(25th-75th percentile)	4 (2.6-5.6)	3.3 (3.2-4)	4 (2.65-5.6)	
Range	0.5-16	2-6.2	0.5-16	

† **Mann Whitney test,** ‡ **Fisher's exact test**

Distribution of ER alpha positive status was comparable in different sizes of tumors. (p value=0.736)

Table 36:-Association of size(cm) with ER beta.

Size(cm)	Negative(n=64)	Positive(n=68)	Total	P value
<3 cm	15 (41.67%)	21 (58.33%)	36 (100%)	0.03§
3 to 5 cm	20 (39.22%)	31 (60.78%)	51 (100%)	
>5 cm	29 (64.44%)	16 (35.56%)	45 (100%)	
Mean ± SD	4.63 ± 2.15	3.96 ± 2.44	4.29 ± 2.32	0.02†
Median(25th-75th percentile)	5 (3.15-6)	3.25 (2.5-5)	4 (2.65-5.6)	
Range	0.5-9	1-16	0.5-16	

† **Mann Whitney test,** § **Chi square test**

Proportion of ER beta positive patients was significantly higher in <3 cm group(58.33%), 3 to 5 cm group (60.78%) as compared to >5 cm group (35.56%). (p value=0.03)

Median(25th-75th percentile) of size(cm) in ER beta negative was 5(3.15-6) which was significantly higher as compared to ER beta positive (3.25(2.5-5)). (p value=0.02)

Table 37:-Association of size(cm) with AR.

Size(cm)	Negative(n=48)	Positive(n=84)	Total	P value
<3 cm	12 (33.33%)	24 (66.67%)	36 (100%)	0.084§
3 to 5 cm	14 (27.45%)	37 (72.55%)	51 (100%)	
>5 cm	22 (48.89%)	23 (51.11%)	45 (100%)	
Mean ± SD	4.52 ± 2.29	4.15 ± 2.33	4.29 ± 2.32	0.244†
Median(25th-75th percentile)	4.5 (2.875-6.275)	3.8 (2.65-5.25)	4 (2.65-5.6)	
Range	0.5-9	1-16	0.5-16	

† **Mann Whitney test,** § **Chi square test**

Distribution of AR positive status was not statistically significant in different sizes of tumors.

Table 38:-Association of multifocality with ER alpha.

Multifocality	Negative(n=127)	Positive(n=5)	Total	P value
No	81 (96.43%)	3 (3.57%)	84 (100%)	1‡
Yes	46 (95.83%)	2 (4.17%)	48 (100%)	
Total	127 (96.21%)	5 (3.79%)	132 (100%)	

‡ **Fisher's exact test**

ER alpha positivity status was not associated with multifocality.

Table 39:-Association of multifocality with ER beta.

Multifocality	Negative(n=64)	Positive(n=68)	Total	P value
No	34 (40.48%)	50 (59.52%)	84 (100%)	0.015§
Yes	30 (62.50%)	18 (37.50%)	48 (100%)	
Total	64 (48.48%)	68 (51.52%)	132 (100%)	

§ **Chi square test**

Proportion of ER beta positive patients was significantly higher in patients with unifocal bladder tumors(59.52%) as compared to patients with multifocality(37.50%). (p value=0.015)

Table 40:-Association of multifocality with AR.

Multifocality	Negative(n=48)	Positive(n=84)	Total	P value
No	29 (34.52%)	55 (65.48%)	84 (100%)	0.561§
Yes	19 (39.58%)	29 (60.42%)	48 (100%)	
Total	48 (36.36%)	84 (63.64%)	132 (100%)	

§ **Chi square test**

Distribution of AR receptors was comparable in patients without and with multifocality. (No(65.48%) vs Yes(60.42%)). (p value=0.561)

Table 41:-Association of metastasis at presentation with ER alpha.

Metastasis at presentation	Negative(n=127)	Positive(n=5)	Total	P value
No	123 (97.62%)	3 (2.38%)	126 (100%)	0.016‡
Yes	4 (66.67%)	2 (33.33%)	6 (100%)	
Total	127 (96.21%)	5 (3.79%)	132 (100%)	

‡ **Fisher's exact test**

ER alpha expression was significantly higher in patients with metastasis at presentation (33.33%) as compared to patients without metastasis at presentation (2.38%). (p value=0.016)

Table 42:-Association of metastasis at presentation with ER beta.

Metastasis at presentation	Negative(n=64)	Positive(n=68)	Total	P value
No	61 (48.41%)	65 (51.59%)	126 (100%)	1‡
Yes	3 (50%)	3 (50%)	6 (100%)	
Total	64 (48.48%)	68 (51.52%)	132 (100%)	

‡ **Fisher's exact test**

Distribution of ER beta positivity was comparable in patients without and with metastasis at presentation. (No(51.59%) vs Yes(50%)). (p value=1)

Table 43:-Association of metastasis at presentation with AR.

Metastasis at presentation	Negative(n=48)	Positive(n=84)	Total	P value
No	47 (37.30%)	79 (62.70%)	126 (100%)	0.416‡
Yes	1 (16.67%)	5 (83.33%)	6 (100%)	
Total	48 (36.36%)	84 (63.64%)	132 (100%)	

‡ **Fisher's exact test**

AR positivity was not found to be associated with metastasis at presentation. (No(62.70%) vs Yes(83.33%)). (p value=0.416)

Table 44:-Association of complications with ER alpha.

Complications	Negative(n=127)	Positive(n=5)	Total	P value
None	108 (96.43%)	4 (3.57%)	112 (100%)	
Abdominal ileus	1 (100%)	0 (0%)	1 (100%)	
AKI	0 (0%)	1 (100%)	1 (100%)	
Anemia	1 (100%)	0 (0%)	1 (100%)	
Cardiac arrest	1 (100%)	0 (0%)	1 (100%)	
Hematuria	5 (100%)	0 (0%)	5 (100%)	
Hypotension	1 (100%)	0 (0%)	1 (100%)	0.345‡
Iatrogenic urinary bladder perforation	4 (100%)	0 (0%)	4 (100%)	
Intestinal obstruction	1 (100%)	0 (0%)	1 (100%)	
Left lower limb DVT postop	1 (100%)	0 (0%)	1 (100%)	
Postop ileus	3 (100%)	0 (0%)	3 (100%)	
Respiratory complications	1 (100%)	0 (0%)	1 (100%)	
Total	127 (96.21%)	5 (3.79%)	132 (100%)	

‡ **Fisher's exact test**

No statistically significant association was found between complications and ER alpha receptor positivity in patients (p value=0.345).

Table 45:-Association of complications with ER beta.

Complications	Negative(n=64)	Positive(n=68)	Total	P value
None	56 (50%)	56 (50%)	112 (100%)	
Abdominal ileus	1 (100%)	0 (0%)	1 (100%)	
AKI	0 (0%)	1 (100%)	1 (100%)	
Anemia	1 (100%)	0 (0%)	1 (100%)	
Cardiac arrest	0 (0%)	1 (100%)	1 (100%)	
Hematuria	0 (0%)	5 (100%)	5 (100%)	
Hypotension	1 (100%)	0 (0%)	1 (100%)	0.098‡
Iatrogenic urinary bladder perforation	1 (25%)	3 (75%)	4 (100%)	
Intestinal obstruction	1 (100%)	0 (0%)	1 (100%)	
Left lower limb DVT postop	1 (100%)	0 (0%)	1 (100%)	
Postop ileus	1 (33.33%)	2 (66.67%)	3 (100%)	
Respiratory complications	1 (100%)	0 (0%)	1 (100%)	
Total	64 (48.48%)	68 (51.52%)	132 (100%)	

‡ **Fisher's exact test**

No statistically significant association was found between complications and ER beta receptor positivity in patients (p value=0.098).

Table 46:-Association of complications with AR.

Complications	Negative(n=48)	Positive(n=84)	Total	P value
None	41 (36.61%)	71 (63.39%)	112 (100%)	0.346‡
Abdominal ileus	0 (0%)	1 (100%)	1 (100%)	
AKI	0 (0%)	1 (100%)	1 (100%)	
Anemia	0 (0%)	1 (100%)	1 (100%)	
Bladder perforation	0 (0%)	1 (100%)	1 (100%)	
Cardiac arrest	0 (0%)	1 (100%)	1 (100%)	
Hematuria	1 (20%)	4 (80%)	5 (100%)	
Hypotension	0 (0%)	1 (100%)	1 (100%)	
Iatrogenic urinary bladder perforation	1 (25%)	3 (75%)	4 (100%)	
Intestinal obstruction	1 (100%)	0 (0%)	1 (100%)	
Left lower limb DVT postop	0 (0%)	1 (100%)	1 (100%)	
Postop ileus	3 (100%)	0 (0%)	3 (100%)	
Respiratory complications	1 (100%)	0 (0%)	1 (100%)	
Total	48 (36.36%)	84 (63.64%)	132 (100%)	

‡ **Fisher's exact test**

No statistically significant association was found between complications and AR receptor positivity in patients (p value=0.346).

Table 47:-Association of length of hospital stay post-surgery with ER alpha.

Length of hospital stay post-surgery	**Negative**	**Positive**	**Total**	**P value**
<3 days	72 (96%)	3 (4%)	75 (100%)	0.725‡
3 to 7 days	33 (94.29%)	2 (5.71%)	35 (100%)	
>7 days	18 (100%)	0 (0%)	18 (100%)	
Death	4 (100%)	0 (0%)	4 (100%)	
Mean ± SD	3.74 ± 3.97	2.8 ± 1.64	3.7 ± 3.91	0.836†
Median(25th-75th percentile)	2 (2-4)	2 (2-4)	2 (2-4)	
Range	1-30	1-5	1-30	

† **Mann Whitney test,** ‡ **Fisher's exact test**

Table 48:-Association of length of hospital stay post-surgery with ER beta.

Length of hospital stay post surgery	**Negative**	**Positive**	**Total**	**P value**
<3 days	37 (49.33%)	38 (50.67%)	75 (100%)	0.239‡
3 to 7 days	13 (37.14%)	22 (62.86%)	35 (100%)	
>7 days	12 (66.67%)	6 (33.33%)	18 (100%)	
Death	2 (50%)	2 (50%)	4 (100%)	
Mean ± SD	3.9 ± 3.88	3.52 ± 3.96	3.7 ± 3.91	0.818†
Median(25th-75th percentile)	2 (2-4)	2 (2-4)	2 (2-4)	
Range	1-20	1-30	1-30	

† **Mann Whitney test,** ‡ **Fisher's exact test**

Table 49:-Association of length of hospital stay post-surgery with AR.

Length of hospital stay post surgery	Negative	Positive	Total	P value
<3 days	26 (34.67%)	49 (65.33%)	75 (100%)	0.464‡
3 to 7 days	12 (34.29%)	23 (65.71%)	35 (100%)	
>7 days	7 (38.89%)	11 (61.11%)	18 (100%)	
Death	3 (75%)	1 (25%)	4 (100%)	
Mean ± SD	4.33 ± 5.41	3.36 ± 2.77	3.7 ± 3.91	0.587†
Median(25th-75th percentile)	2 (2-4)	2 (2-4)	2 (2-4)	
Range	1-30	1-13	1-30	

† **Mann Whitney test,** ‡ **Fisher's exact test**

Distribution of ER alpha, ER beta and AR positivity was not found to be associated with length of hospital stay post-surgery.

Median(25th-75th percentile) of length of hospital stay post-surgery in ER alpha, ER beta and AR negative patients and positive patients had no significant association between them. (Tables 46,47,48)

Table 50:-Association of complications with surgery done.

Complications	Palliative cystectomy(n=2)	Random bladder biopsies(n=1)	RC with IC(n=19)	RC with neobladder(n=1)	Restage TURBT(n=1)	TUR biopsy (n=17)	TURBT(n=91)	Total	P value
None	2 (100%)	1	11	0 (0%)	1	15	82	112	0.0

		(100%)	(57.89%)		(100%)	(88.24%)	(90.11%)	(84.85%)	07‡
Abdominal ileus	0 (0%)	0 (0%)	1 (5.26%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.76%)	
AKI	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.10%)	1 (0.76%)	
Anemia	0 (0%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	0 (0%)	1 (0.76%)	
Bladder perforation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.10%)	1 (0.76%)	
Cardiac arrest	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1.10%)	1 (0.76%)	
Hematuria	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.88%)	4 (4.40%)	5 (3.79%)	
Hypotension	0 (0%)	0 (0%)	1 (5.26%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.76%)	
Latrogenic urinary bladder perforation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (5.88%)	2 (2.20%)	3 (2.27%)	
Intestinal obstruct	0 (0%)	0 (0%)	1 (5.26%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.76%)	

ion								
Left lower limb DVT postop	0 (0%)	0 (0%)	1 (5.26 %)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.7 6%)
Postop ileus	0 (0%)	0 (0%)	3 (15.7 9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (2.2 7%)
Respira tory complic ations	0 (0%)	0 (0%)	1 (5.26 %)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (0.7 6%)
Total	2 (100%)	1 (100%)	19 (100 %)	1 (100%)	1 (100%)	17 (100%)	91 (100%)	132 (100 %)	

‡ **Fisher's exact test**

Patients who underwent Palliative cystectomy, Random bladder biopsies and Restage TURBT had no complications. On the other hand, out of 19 patients who underwent RC with IC, 11 (57.89%) patients had no complications, 1 (5.26%) patient had abdominal ileus, hypotension, intestinal obstruction, left lower limb DVT postop and respiratory complications each and 3 (15.79%) patients had postop ileus. 1 patient of RC with neobladder had anemia. 1 (5.88%) patient of TUR biopsy had hematuria and latrogenic urinary bladder perforation each. 4 (4.40%) patients of TURBT had hematuria, 2 (2.20%) patients had latrogenic urinary bladder perforation and 1 (1.10%) patient had bladder perforation, AKI, cardiac arrest each. So distribution of complications among various surgeries was significantly different. (p value=0.007)

Table 51:-Association of ER alpha with ER beta.

ER alpha	ER beta		Total	P value
	Negative(n=64)	Positive(n=68)		
Negative	63 (98.44%)	64 (94.12%)	127 (96.21%)	0.367‡
Positive	1 (1.56%)	4 (5.88%)	5 (3.79%)	
Total	64 (100%)	68 (100%)	132 (100%)	

‡ **Fisher's exact test**

Distribution of ER alpha was comparable between negative and positive ER beta. (ER alpha negative:- 98.44% vs 94.12% respectively, ER alpha positive:- 1.56% vs 5.88% respectively) (p value=0.367).

Table 52:-Association of ER alpha with AR.

ER alpha	AR		Total	P value
	Negative(n=48)	Positive(n=84)		
Negative	47 (97.92%)	80 (95.24%)	127 (96.21%)	0.653‡
Positive	1 (2.08%)	4 (4.76%)	5 (3.79%)	
Total	48 (100%)	84 (100%)	132 (100%)	

‡ **Fisher's exact test**

Distribution of ER alpha was comparable between negative and positive AR. (ER alpha negative:- 97.92% vs 95.24% respectively, ER alpha positive:- 2.08% vs 4.76% respectively) (p value=0.653).

Table 53:-Association of ER beta with AR.

ER beta	AR		Total	P value
	Negative(n=48)	Positive(n=84)		
Negative	27 (56.25%)	37 (44.05%)	64 (48.48%)	0.177§
Positive	21 (43.75%)	47 (55.95%)	68 (51.52%)	
Total	48 (100%)	84 (100%)	132 (100%)	

§ **Chi square test**

Distribution of ER beta was comparable between negative and positive AR. (ER beta negative:- 56.25% vs 44.05% respectively, ER beta positive:- 43.75% vs 55.95% respectively) (p value=0.177).

DISCUSSION

DISCUSSION

Urinary bladder carcinoma is the fourth most common malignancy in males and eleventh most common one in females(14). It is a malignancy with heterogeneous presentation, diverse biological behaviour and sometimes an unpredictable response to available therapeutic modalities. Looking at these features, its high progression rate on existing treatment modalities and resulting significant mortality, the quest for new therapeutic targets continues. Androgen and estrogen receptors have recently been explored as a promising target for treatment of bladder cancer patients. The gender disparity in incidence as well as the association of more aggressive bladder malignancies in females has further raised the question of relationship of sex steroid receptor expression and gender. In our study, 132 patients who underwent surgical management for bladder cancer and have not received any previous chemotherapeutic treatment were included from a time period of january 2019 to january 2022. Their tumor blocks were analyzed for 3 prominent steroid receptors- ER alpha, ER beta and AR. 116(87.88%) patients were males and 16(12.12%) patients were females. Most of the patients i.e. 42(31.82%) belonged to the age group of 61-70 years followed by 51-60 years (28.79%). The Histologic stage and grade of maximum patients (31.82%) was high grade with invasion to lamina propria (T1G3) followed by muscle invasive disease (T2) in 29.55% of patients. Distant metastases at presentation was there in only 6 of 132 patients. Majority of patients had tumors of size >3cm, with unifocal tumors in majority. Pure urothelial variant of bladder cancer was found in >80% of tumors.

A study by Mashhadi et al (62) had 85.7% of patients as male. In the study conducted by Miyamato et al, 148 patients were males and 40 were females. Nam et al showed similar gender distribution, supporting the male predominance of the disease. Gupta et al(15) found male to female ratio of bladder cancer to be 8.6:1.

Bladder cancer is rare in people younger than 50 years of age, even though it can occur at any age. The incidence of malignancy is directly proportional to age with the median age at diagnosis to be about 70 years for both genders(16). Mashhadi et al(62) also found the mean age to be 66.2 ± 12.10 years of cancer patients in their study, which is similar to ours. Gupta et al(15) stated that the median age of presentation in bladder cancer is 69 years old for males and 71 years old for females. In studies of Miyamato et al and Wagih et al, maximum patients had T2 and T3 disease, in contrast to our study where >60% of patients were of T1G3 and T2 stage, with just 5 out of 132 presenting with T3 disease. This shows improving

awareness and better accessibility to health care facilities, leading to presentation at a lower disease stage. Gupta et al(15) also found that in the Indian population about 40 to 45% of bladder malignancies are high-grade at presentation, with more than half of them being muscle invasive.

The incidence of bladder cancer is higher in men than women. One of the possible reasons for the variation observed is due to differences in occupational exposure and smoking. However, animal studies have shown that along with malignancies that are chemically induced,even bladder malignancies developing spontaneously are significantly higher in male rats than females. Also it has been seen that incidence of bladder cancer in postmenopausal women is more common than women who are premenopausal. The bladder tumors possessing androgen receptors were found to have a positive stimulus on growth due to circulating androgens. In another study, it has been found that antiestrogens can inhibit urothelial carcinoma of the bladder in ER-positive bladder cancer cell lines. In contrast to the above mentioned hypothesis that there might be a role of androgen and estrogen receptor expression in male predominance of bladder cancer, no statistically significant association was found between expression of these receptors and gender. This finding is supported by results of studies by Miyamoto et al and Nam et al. However, Ide et al.(21) in their metanalysis showed that AR expression was down-regulated in female patients in comparison to male patients of ca bladder (P = 0.027)

Only 5 out of 132 patients (3.79%) showed ER alpha positivity in our study. This is close to the results of a study by Bolenz et al (57) who found ER alpha positivity in nine of the 198 specimens (4.5%). In contrast, Miyamoto et al (59) showed ER alpha positivity in 51 out of 188 (27%) primary tumors. In our study, 51.52% of patients had ER beta positivity. This was close to results of Miyamoto et al in which ER beta positivity was there in 93 out of 188 (49%) primary tumors. As far as AR expression is concerned, different studies showed different results. In our study, AR positivity was seen in 63.64% of patients. Our findings were close in this respect to findings of Wagih et al who found expression of AR in 58% of patients. However, different results were described by Nam et al., Boorjian et al.,Mashhadi et al. and Miyamoto et al., who showed the expression of AR in 37% , 53.1% , 22% and 42% of the bladder cancer patients, respectively.

Distribution of ER alpha, ER beta and AR positivity was comparable in all age groups and their correlation was not statistically significant in our study. Miyamoto et al stated that

patients with AR-positive tumors tended to be older than those with AR-negative tumors. However, this finding of theirs was not statistically significant (P = 0.0738). In contrast, Pachauri et al (28) found that AR positivity was significantly (P = 0.02) lower among the patients of age <50 years (7.1%) than ≥50 years (50%).

No statistically significant correlation was found between ER alpha positive status and tumor stage and grade in our study.(p value=0.719). This finding is contrasting to other studies, like Miyamoto et al. who found a lower expression of ERα in high grade and muscle invasive tumors than in low grade tumours.(59) Similar results were found by Bolenz et al(57). Ide et al(21) found expression of ERα to be downregulated significantly in malignant tumors, compared with non-tumors (P < 0.001).

In our study, distribution of ER beta positivity was comparable in various histologic stages and grades and no statistically significant difference was found in their expression. This is again contradictory to what has been stated by Miyamoto et al(59) and metanalysis by Ide et al (21) that there is higher expression of ER beta in tumors with higher grade and stage.

We found no statistically significant correlation was found between AR positive status and tumor stage and grade.(p value=0.214) This result is supported by studies done by Wagih et al and Pachauri et al. The notable point in this respect is that many previous studies which evaluated the correlation between AR and bladder tumor aggressiveness stated conflicting results. For example, Tuygun et al. (61) reported an inverse relationship between AR expression and tumors with higher grades and stage, which is consistent with the findings of Miyamoto et al and Boorjian et al. (17). In contrast, Mir et al. (62) showed that AR expression was greater in muscle invasive tumors (15%) compared to non- invasive ones (9%). Similarly Mashhadi et al. (63) also found direct correlation between AR expression and invasiveness of bladder tumor. Such discrepancies between studies may be due to variation in staining conditions, difference in criterias for receptor expression or difference in study population characteristics.

This discrepancy in results obtained can be attributed to complex nature of the bladder neoplasms, leading to certain unavoidable confounders and effect modifiers that might interfere with the obtained results(62).

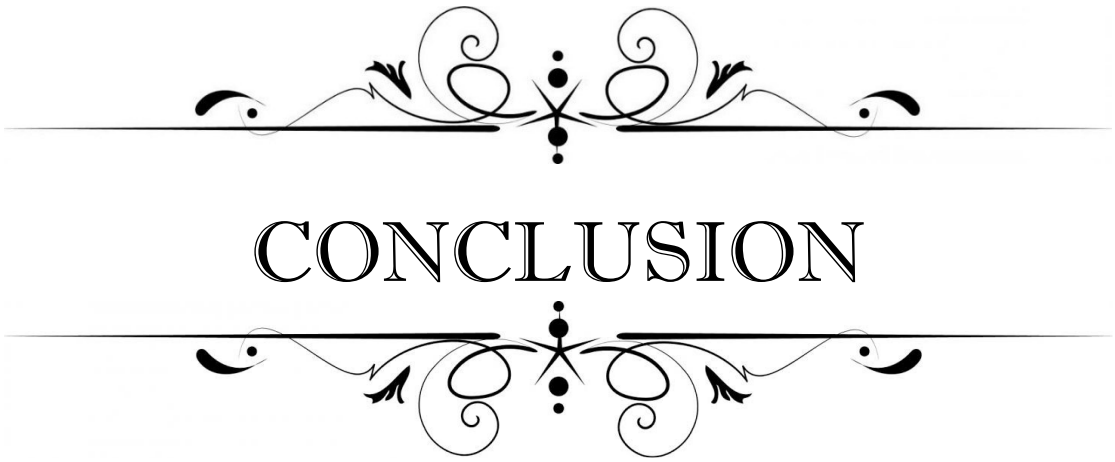
Our study was one of its kind to correlate presence of pathologic variants, size, multifocalily and postoperative complications with expression of AR and ER. On correlating expression of

all 3 receptors in different pathologic variants and found no statistically significant correlation.

We studied the relationship between expression of sex steroid receptors and various clinical parameters in our study patients. As far as the expression of the sex steroid receptors and their relationship with size of bladder tumors and multifocality is concerned, comparable results were found with ER alpha and AR. But the proportion of ER beta positive patients was significantly higher in <3 cm group (58.33%), 3 to 5 cm group (60.78%) as compared to >5 cm group (35.56%). (p value=0.03). Also, proportion of ER beta positive patients was significantly higher in patients with unifocal bladder tumors(59.52%) as compared to patients with multifocality(37.50%). (p value=0.015)

ER alpha expression was significantly higher in patients with metastasis at presentation (33.33%) as compared to patients without metastasis at presentation (2.38%). (p value=0.016) this finding is contrasting to results of other studies such as Croft et al. who found all metastatic foci are negative of ERα expression in bladder cancer patients(58). Miyamoto et al and Bolenz et al also inversely correlated ER alpha expression with aggressiveness of bladder tumor. Distribution of ER beta and AR positivity was comparable in patients without and with metastasis at presentation.

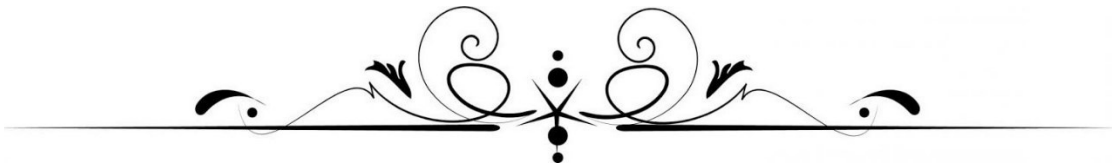
No statistically significant association was found between complications, length of hospital stay and ER alpha, ER beta and AR receptor positivity in patients. We also correlated expressions of ER alpha, ER beta and AR with each other but found it to be insignificant.



CONCLUSION

CONCLUSION

In the available literature, there has been great discordance in androgen and estrogen receptor expression and relationship with various clinicopathologic features. Our study is the one with the largest sample size conducted on the Indian population, and it revealed results which differ in various aspects from other similar studies of the past. Though AR and ER beta expression is high in bladder tumors and ER alpha expression is positive in a low percentage of patients which is similar to various other studies, no correlation has been found between their expression and tumor stage and grade.The expression of ER beta was more in small sized unifocal tumors, which was contradictory to other studies showing direct relationship between ER beta and tumor invasiveness. ER alpha expression was found to be higher in metastatic tumors, while other studies found its greater expression in low grade tumors. Such great variation shows that further studies are required on a larger scale from different geographic areas to have an actual understanding of biological behaviour of sex steroid receptors in bladder cancer and to answer the question of judicious implication of therapeutic agents targeting these receptors for benefitting patients who doesnot respond to or progress on conventional therapeutic modalities.



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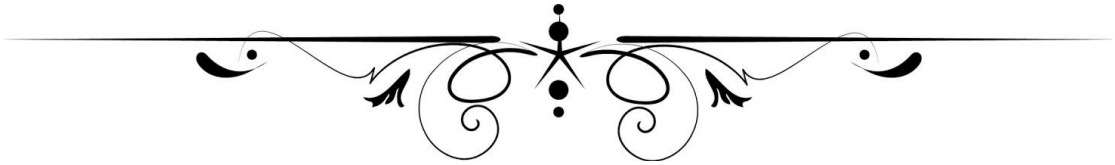

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ANNEXURES

ANNEXURE-I

Ethical Clearance Certificate

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/3325 Date: 14/10/2020

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/963**

Project title: **"Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features"**

Nature of Project:	**Research Project**
Submitted as:	**M.Ch. Dissertation**
Student Name:	**Dr.Nikita Shrivastava**
Guide:	**Dr.Himanshu Pandey**
Co-Guide:	**Dr. Gautam Ram Choudhary, Dr. Mahendra Singh, Dr. Vijay Kumar Sarma Madduri, Dr. Poonam Elhence, Dr. Vikarn Vishwajeet & Dr. Arvind Sinha**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary
Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
E-mail : ethicscommittee@aiimsjodhpur.edu.in; ethicscommitteeaiimsjdh@gmail.com

ANNEXURE-II A

CONSENT FORM

I_____S/O,W/O,D/O_____

___R/O_____

_____,Exercising my free power of choice, hereby give my consent to be included as a subject in the study entitled : Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features"

I have been given a full explanation by the study doctor of the nature, purpose and the likely duration of the study and what I will be expected to do and I have been advised any foreseeable risk associated with the procedure. This has been explained to me in the language I best understand.

I agree to cooperate fully with the supervising doctor and to inform him/her immediately if Isuffer from any unusual symptoms during the study period. I am also aware of my right to opt out at any stage of the trial during the course of study and my usual treatment will be continue. I understand that medical records that reveal my identity will be kept confidential.

Name of the patient: Name of supervising doctor

Signature: Dr. Nikita Shrivastava

Name of accompanying relative:

Signature:

ANNEXURE-II B

सहमतिपत्र

मैं_____**पत्नी/बेटी/पति/बेटा**_____

_____**निवासी**_____

_____ **अपनी इच्छा अनुसार** Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features" **नामकशोध अध्ययन में एक अध्ययन विषय के रूप में शामिल होने के लिए अपनी पूर्ण स्वतंत्र और स्वैच्छिक सहमति देता / देती हूँ । मुझे पूर्ण संतुष्टि अनुसार ड्यूटी पे तैनात चिकित्सक द्वारा अध्ययन के उद्देश्य के बारे मैं स्पष्ट स्पष्ट बता दिया गया हैं। मुझे बता दिया गया हैं की इस शोधकार्य में मेरी भागीदारी पूरी तरह स्वैछिक है और मैं किसी भी दण्ड के भय के बिना और ऐसा करने के मेरे कारण को दिए बिना किसी भी समय इस अध्ययन से अपनी भागीदारी समाप्त करने के लिए चुन सकता / सकती हूँ। मैं इस शोध कार्य में भाग लेने के लिए किसी भी मौद्रिकयावित्तीय या मुआवज़े के किसी अन्यरूप का दावा नहीं करूंगा / करुंगी । मैं समझती हूँ की मेरी बिमारी की जानकारी एवं उस सेसम्बंधित सभी वस्तुएं गोपनीय रखी जाएँगी लेकिन रोगी की गोपनीयता अनुसंधानकाविशेय है और अन्य स्वास्थ्य सेवा प्रदाताओं की रक्षा हेतु इसे तोड़ा जा सकता है।**

मैं अपने हस्ताक्षर द्वारा इसशोध कार्य मैं पूरा सहयोग एवं बिमारी से सम्बंधित जानकारी डॉक्टर के साथ बाटने का वादा करता / करती हूँ।

मरीज़ का नाम: **मरीज़के हस्ताक्षर**

तिथि :

पर्यवेक्षक: डॉ। निकिता श्रीवास्तव

यूरोलॉजी विभाग, एम्स जोधपुर

तिथि : **गवाह** 1 **का नाम** : **गवाह** 1 **के हस्ताक्षर**

ANNEXURE-III A

PATIENT INFORMATION FORM

Patient Identification Number for this research project:

Title : Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features**"**

Student : Dr. NIKITA SHRIVASTAVA

Supervisor : Dr. GAUTAM RAM CHOUDHARY

Associate Professor

Department of Urology, AIIMS Jodhpur

You are being invited to participate in this research to assess: **"** Androgen receptor and estrogen receptor expression in carcinoma urinary bladder and correlation with clinicopathologic features**"**

PURPOSE OF RESEARCH: Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features**"**

EXPECTED DURATION OF PARTICIPATION: Patient's follow up will be divided in to : 1) short duration follow up (due to restricted study duration) and 2) long duration follow up till death of patient or lost to follow up.

FORESEEABLE RISKS BY PARTICIPATING IN THE STUDY: Patient will undergo normal treatment protocol ,investigations and surgeries risks will be involved but there is no extra risk due to participation in this study.

BENEFITS BY PARTICIPATING IN THE STUDY: No direct benefit would be there as far as the present study is concerned. However, it may beneficial to the society and the other people if this study will able to help in modifying the management of patients.

ALTERNATIVES TO PARTICIPATION: None, as this is an observational study.

CONFIDENTIALITY: All the information that you or your patient provides during the study will be kept confidential on a password protected computer. Information collected about the patient from his/her participation in this research and sections of any of his/her medical notes will not be used for any other purpose but it may be looked at by responsible individuals.

COST OF PARTICIPATION: No additional cost to you for participating in this study.

PAYMENT FOR PARTICIPATION: No incentives to you for participating in this study.

In the event that at any time during the course of the study you/your patient feels that you/they have not been adequately informed as to the possible risks, benefits, alternative procedures, or rights as a study subject or feel under pressure to continue against your wish you can contact:

principal investigator :

Dr. NIKITA SHRIVASTAVA
Department of Urology,
AIIMS Jodhpur, 342005
Tel: 7225839193

Dr. GAUTAM RAM CHOUDHARY
Associate Professor,
Department of Urology,
AIIMS Jodhpur, 342005

LEGAL RIGHTS: By signing this form, we are not violating any of your legal rights.
The patient or patient's relative will be notified in a timely manner if significant new findings develop during the course of the research which may affect the subject's willingness to continue participation.

Date:

Place:

Signature/left thumb impression of the patient: _____

Patient's name:_____

Signature of principal investigator:
Date:

ANNEXURE-III B

रोगी सूचना पत्र

इस शोध परियोजना के लिए रोगी पहचान संख्या:

शीर्षक Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features"

छात्र: **डॉ। निकिता श्रीवास्तव**

पर्यवेक्षक: **डॉ गौतम राम चौधरी**

सह - प्राध्यापक

यूरोलॉजी विभाग, एम्स जोधपुर

मूल्यांकन के लिए आपको इस शोध में भाग लेने के लिए आमंत्रित किया जा रहा है:

Androgen and estrogen receptor expression in carcinoma urinary bladder and relationship with clinicopathologic features"

भागीदारी की अपेक्षित अवधि: रोगी का अनुवर्ती भाग इस प्रकार विभाजित किया जाएगा:

1) **छोटी अवधि अनुवर्ती** (**प्रतिबंधितअध्ययनअवधिकेकारण**) **और**

2) **लंबे समय तक रोगी की मौत तक फॉलोअप या फॉलोअप के लिए खो दिया।**

अध्ययन में भाग लेने के लिए जोखिम : आपके इलाज में होने वाले जोखिम से इस जांच का कोई नाता नहीं है. इसमें किसी भी प्रकार का अलग से जोखिम नहीं है .

अध्ययन में भाग लेने से लाभ:वर्तमान अध्ययन के संबंध में कोई प्रत्यक्ष लाभ नहीं होगा । हालांकि, यह समाज और अन्य लोगों के लिए फायदे मंद हो सकता है य दियi ह अध्ययन रोगियों के प्रबंधनमें संशोधन करने में मदद करेगा।

समूह आवंटन:कोई नहीं

साझेदारी के लिए विकल्प:कोई नहीं, क्योंकि यह एक अवलोकन अध्ययन है।

गोपनीयता:अध्ययन के दौरान आप या आप के रोगी द्वारा प्रदान की जाने वाली सभी जानकारी को पास वर्ड संरक्षित कंप्यूटर पर गोपनीय रखा जाएगा ।इस शोध में रोगी के बारे में एकत्रित जानकारी और उसके किसी भी चिकित्सा नोट के अनुभागों का उपयोग किसी भी अन्य उद्देश्य के लिए नहीं किया जाएगा, लेकिन इसे जिम्मेदार व्यक्तियों द्वारा देखा जा सकता है।

साझेदारी की लागत:इस अध्ययन में भाग लेने के लिए आपके लिए कोई अतिरिक्त लागत नहीं है।

भागीदारी के लिए भुगतान: इस अध्ययन में भाग लेने के लिए आपके लिए कोई प्रोत्साहन नहीं।

यदि किसी भी समय अध्ययन के दौरान आप / आप के रोगी को लगता है कि आपको संभावित जोखिम, लाभ, वैकल्पिक प्रक्रियाओ या अध्ययन विषय के रूप में अधिकारों के रूप में पर्याप्त रूप से सूचित नहीं किया गया है या जारी रखने के दबाव में महसूस नही किया गया है आपकी इच्छा के विरुद्ध आप संपर्क कर सकतेहैं।

डॉ। निकिता श्रीवास्तव
यूरोलॉजी विभाग,
एम्स जोधपुर, 342005
दूरभाष: 7225839193

डॉ गौतम राम चौधरी
एसोसिएट प्रोफेसर,
यूरोलॉजी विभाग,
एम्सजोधपुर, 342005

कानूनी अधिकार :इस फॉर्म प रहस्ताक्षर करके, हम आपके किसी भी कानूनी अधिकार का उल्लंघन नहीं कर रहे हैं।

रोगी या रोगी के रिश्तेदार को समय-समय पर अधिसूचित किया जाएगा यदि अनुसंधान के दौरान महत्वपूर्ण नए निष्कर्ष विकसित होते हैं जोकि विषय जारी रखने की इच्छा को प्रभावित कर सकते हैं।

दिनांक:

जगह:

रोगीकेहस्ताक्षर / बाएंअंगूठेकीछाप: _____

मरीज का नाम:_____

प्रिंसिपल अन्वेषक का हस्ताक्षर:

दिनांक:

सह-जांचकर्ता का हस्ताक्षर:

दिनांक:

ANNEXURE-IV

PROFORMA

Name:

Age: Sex:

CR Number:

Address with phone number:

addiction:

Comorbidity:

Chief complaints and duration:

General and systemic examination:

Date of admission:

Date of surgery:

Radiological findings:

USG KUB :

CT UROGRAPHY:

MR urography:

Surgery details:

D) Perioperative parameters(for prospective cases only):

1. complications (if any)

2. degree of haematuria post surgery

3. length of hospital stay

histopathologic report:

Tumor type

Tumor stage

Tumor grade

Immunohistochemical assay

Androgen receptor

Estrogen receptor alpha

Estrogen receptor beta

ANNEXURE V

MASTERCHART

	Age	Gender	Histologic stage and grade	Pathological type	Size of mass						ER alpha	ER beta	AR
					Size in cm	Focality	Metastases at presentation	Surgery done	complications	length of hospital stay post surgery	positive/negative	positive/negative	positive/negative
AIIMS/JDH/2019/03/011804	49	F	T1G3	Urothelial	2	-	No	TURBT	None	2 days	N	N	P
AIIMS/JDH/2020/01/029056	60	M	T1G3	Urothelial	3.5	-	No	TUR biopsy	None	2 days	N	N	P
AIIMS/JDH/2019/12/006933	62	M	T1G1	Urothelial	5.4	Multifocal	No	TUR biopsy	None	1 day	N	N	P
AIIMS/JDH/2020/09/009741	72	M	TaG1	Urothelial	4.7	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2020/01/023263	73	M	T1G3	Chondrosarcomatous	5.2	-	No	TURBT	None	2 days	N	N	P
AIIMS/JDH/2020/11/001274	31	F	TaG1	Urothelial	1.5	-	No	TURBT	None	1 day	N	P	N
AIIMS/JDH/2019/09/019641	40	M	T1G3	Urothelial	2.5	-	No	TURBT	None	1 day	N	P	P
AIIMS/JDH/2020/10/009512	60	M	T2	Urothelial	4.5	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2019/06/010274	39	M	T2	Urothelial	1.3	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2020/01/021310	43	M	TaG3	Urothelial	3.1	Multifocal	No	TURBT	Hematuria	2 days	N	P	P
AIIMS/JDH/2021/02/000196	66	M	T3b	Urothelial	6	Multifocal	Yes	Palliative cystectomy	None	10 days	N	N	P
AIIMS/JDH/2021/02/002667	49	M	TaG1	Urothelial	1	-	No	TURBT	None	1 day	N	N	P
AIIMS/JDH/2019/03/015938	44	M	TaG1	Urothelial	6.7	-	No	RC with IC	None	9 days	N	N	P
AIIMS/JDH/2021/01/017187	56	M	T2	Squamous	5	-	No	TURBT	None	1 day	N	N	P
AIIMS/JDH/2016/06/013809	69	M	T1G3	Urothelial	4.7	-	No	TURBT	None	1 day	N	N	P
AIIMS/JDH/2018/11/015362	80	M	TaG1	Urothelial	16	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2019/07/021743	57	F	T2	Urothelial	4.8	Multifocal	No	RC with IC	Left lower limb DVT postop	9 days	N	N	P
AIIMS/JDH/2019/06/017207	48	M	T1G3	Urothelial	2.3	-	No	RC with IC	None	10 days	N	N	P
AIIMS/JDH/2020/10/000461	77	M	T1G1	Squamous	3	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2020/10/001933	78	M	T2	Urothelial	4.3	-	No	TURBT	None	2 days	N	N	P
AIIMS/JDH/2020/09/008379	65	M	T1G3	Squamous	5.5	-	No	TURBT	None	2 days	N	P	N
AIIMS/JDH/2020/10/006335	60	M	T2	Squamous	3.8	-	Yes	TURBT	None	2 days	N	P	P
AIIMS/JDH/2020/02/003852	70	M	T1G3	Urothelial	3.2	Multifocal	Yes	TURBT	None	2 days	N	N	P
AIIMS/JDH/2021/01/017387	71	M	T4a	Urothelial	2.7	Multifocal	No	RC with IC	Hypotension	11 days	N	N	P
AIIMS/JDH/2015/03/007563	58	M	T1G3	Urothelial	6.2	-	No	TURBT	None	2 days	N	N	P
AIIMS/JDH/2019/06/004232	60	M	T1G3	Urothelial	2.4	Multifocal	No	RC with IC	None	6 days	N	P	P
AIIMS/JDH/2021/05/000106	65	M	T2	Small round blue cell tumor	5.6	-	No	TUR biopsy	iatrogenic urinary bladder perforation	4 days	N	P	P
AIIMS/JDH/2021/07/000957	51	M	TaG1	Urothelial	3	-	No	TURBT	none	2 days	N	P	P
AIIMS/JDH/2021/02/006913	56	M	T1G3	Urothelial	1.7	Multifocal	No	TURBT	NONE	2 DAYS	N	N	P
AIIMS/JDH/2019/11/009501	101	M	T2	Urothelial	5.6	-	No	TUR biopsy	none	3 days	N	N	P
AIIMS/JDH/2021/02/009706	71	M	T1G3	Urothelial	1.1	-	No	TURBT	none	3 days	N	N	P
AIIMS/JDH/2021/03/001845	75	M	TaG1	Urothelial	5.5	Multifocal	No	TURBT	none	3 days	N	P	P
AIIMS/JDH/2021/02/000196	66	M	T1G3	Urothelial	3.4	Multifocal	No	TUR biopsy	none	4 days	N	P	P
AIIMS/JDH/2017/03/013040	60	M	T2	Squamous	7.5	Multifocal	No	TUR biopsy	HEMATURIA	5 DAYS	N	P	P
AIIMS/JDH/2020/12/007962	71	F	T2	Urothelial	2	-	No	TURBT	AKI	5 DAYS	P	P	P
AIIMS/JDH/2021/04/008747	53	M	T1G3	Urothelial	7.5	-	No	TUR biopsy	none	1 DAY	N	N	P
AIIMS/JDH/2021/04/012052	53	F	T1G3	Urothelial	3.3	Multifocal	Yes	TUR biopsy	none	2 days	P	P	P
AIIMS/JDH/2021/05/008020	55	M	TaG1	Urothelial	9	Multifocal	No	TUR biopsy	none	5 days	N	N	P
AIIMS/JDH/2021/07/012811	55	M	T1G1	Urothelial	3.2	-	No	TURBT	none	4 days	P	P	P
AIIMS/JDH/2019/12/001224	64	M	T2	Urothelial	4	-	No	TURBT	HEMATURIA	2 days	N	P	P
AIIMS/JDH/2019/01/026053	42	M	T1G1	Urothelial	2.9	-	No	TURBT	iatrogenic urinary bladder perforation	2 days	N	N	P
AIIMS/JDH/2019/12/006933	62	M	TaG1	Urothelial	5.2	Multifocal	No	RC with neobladder	anemia	13 days	N	N	P
AIIMS/JDH/2019/12/008098	84	M	T1G3	Urothelial	1.3	-	No	TURBT	none	2 days	N	P	P
AIIMS/JDH/2019/08/012542	60	M	T1G1	Urothelial	7.4	Multifocal	No	RC with IC	Respiratory complications	Death	N	N	N
AIIMS/JDH/2017/04/011083	71	M	T1G3	Urothelial	3.2	Multifocal	No	RC with IC	abdominal ileus	8 days	N	N	P
AIIMS/JDH/2021/07/016626	55	M	T2	Urothelial	6.2	Multifocal	Yes	TURBT	None	2 days	P	N	N
AIIMS/JDH/2019/04/013157Narayan Das	58	M	T1G3	Urothelial	1.4	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2021/10/004304	65	M	T1G3	Plasmacytoid	2	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2015/07/011927	64	M	T2	Urothelial	3	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2017/12/006059	80	M	T1G3	Squamous	5	Multifocal	No	TUR biopsy	None	2 days	N	N	P
AIIMS/JDH/2019/04/010164Multan RamH/3072 /2019	70	M	T3b	Urothelial	7	Multifocal	No	RC with IC	None	11 DAYS	N	P	N
AIIMS/JDH/2017/03/013040	60	M	T2	Squamous	6.5	Multifocal	No	palliative cystectomy	None	5 days	N	P	N
AIIMS/JDH/2018/12/011563	70	M	T1G3	Urothelial	1	Multifocal	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2020/06/003104	33	F	T2	Urothelial	3.5	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2020/05/001270	76	M	T1G3	Squamous	4.8	-	No	TUR biopsy	None	3 days	N	P	P
AIIMS/JDH/2021/07/013104	69	M	T1G3	Urothelial	4	-	No	TURBT	None	1 day	P	P	P
AIIMS/JDH/2021/08/002648	70	M	T1G3	Urothelial	3.2	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2021/08/017362	63	M	TaG1	Urothelial	3	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2021/09/005200	44	M	T1G3	Urothelial	7.5	Multifocal	No	TURBT	None	3 days	N	N	N
AIIMS/JDH/2021/10/006545	63	M	T2	Urothelial	4	-	No	TURBT	None	2 days	N	N	P
AIIMS/JDH/2021/07/013203	80	M	TaG1	Urothelial	0.5	-	No	TURBT	None	3 days	N	N	N
AIIMS/JDH/2020/08/007077	70	F	T2	Urothelial	7.2	Multifocal	No	RC with IC	None	6 days	N	P	N
AIIMS/JDH/2021/08/014214	44	M	T3b	Urothelial	6.5	Multifocal	No	RC with IC	Wound dehiscence	1 month	N	P	N
AIIMS/JDH/2019/01/031600	56	M	T1G3	Urothelial	4.5	-	No	TURBT	None	1 day	N	P	N
AIIMS/JDH/2019/04/014329	58	M	TaG1	Urothelial	1.2	Multifocal	No	Restage TURBT	None	3 days	N	N	P
AIIMS/JDH/2019/04/000684	80	M	T2	Adenocarcinoma	3.2	Multifocal	No	TUR biopsy	None	4 days	N	N	N
AIIMS/JDH/2019/04/018980	49	M	T2	Squamous	6	-	No	TURBT	None	3 days	N	N	N
AIIMS/JDH/2019/07/008711	71	M	T3b	Squamous	6.8	Multifocal	No	RC with IC	fever	Death	N	N	N
AIIMS/JDH/2019/06/005665	52	F	T2	Urothelial	5.5	Multifocal	No	RC with IC	Intestinal obstruction	20 days	N	N	N
AIIMS/JDH/2014/06/004285	76	M	T1G3	Urothelial	4	-	No	TURBT	None	3 days	N	N	P
AIIMS/JDH/2019/09/015451	54	M	TaG1	Urothelial	1.5	-	No	TURBT	None	1 day	N	N	N
AIIMS/JDH/2019/09/016171	59	M	T1G3	Adenocarcinoma	6	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2019/06/008703	69	M	T1G3	Urothelial	6	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2019/11/014293	90	M	T2	Urothelial	5	-	No	TURBT	HEMATURIA	10 days	N	P	N
AIIMS/JDH/2019/11/017076	54	M	T1G1	Urothelial	6	-	No	TURBT	None	1 day	N	P	P
AIIMS/JDH/2021/01/013166	60	M	T2	Urothelial	7.5	Multifocal	No	TURBT	None	2 days	N	N	P
AIIMS/JDH/2017/06/002991	83	M	T1G1	Urothelial	3	-	No	TURBT	None	2 days	N	P	N
AIIMS/JDH/2019/04/004384	57	M	T2	Urothelial	8	Multifocal	No	RC with IC	None	8 days	N	N	N
AIIMS/JDH/2018/11/002166	49	M	TaG1	Urothelial	1.5	Multifocal	No	TURBT	None	5 days	N	P	P
AIIMS/JDH/2016/08/010860	67	M	TaG1	Urothelial	2	-	No	TURBT	None	1 day	N	N	N
AIIMS/JDH/2018/10/014191	60	M	T1G1	Urothelial	5.5	Multifocal	No	TUR biopsy	None	4 days	N	N	P
AIIMS/JDH/2020/12/007314	60	M	T2	Urothelial	6.5	Multifocal	No	RC with IC	None	13 days	N	N	N
AIIMS/JDH/2021/10/014174	60	M	TaG1	Urothelial	1.8	Multifocal	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2021/07/002027	68	M	T1G3	Squamous	6	-	No	TURBT	Bladder perforation	4 days	N	P	P
AIIMS/JDH/2021/08/000523	70	M	T2	Urothelial	5	-	No	TURBT	None	9 days	N	P	P
AIIMS/JDH/2021/10/008047	38	F	T2	Urothelial	5	Multifocal	No	TURBT	None	4 days	N	N	P
AIIMS/JDH/2017/01/015256	69	M	T2	Urothelial	4.2	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2018/11/003755	49	M	TIG3	Urothelial	10	multifocal	No	RC with IC	None	8 days	N	P	P
AIIMS/JDH/2017/10/009428	62	M	TIG3	Urothelial	3.5	-	No	TURBT	None	3 days	N	P	P
AIIMS/JDH/2018/02/005301	65	F	TIG3	Urothelial	2.8	-	No	TURBT	None	3 days	N	P	P
AIIMS/JDH/2018/10/014234Moda Ram	76	M	T2	adenocarcinoma	4.3	-	No	TURBT	None	2 days	N	N	P
AIIMS/JDH/2017/06/009487	55	M	T1G1	Urothelial	8.5	multifocal	No	RC with IC	None	11 days	N	N	P
AIIMS/JDH/2017/09/009140	78	M	TIG3	Urothelial	4.5	multifocal	YES	TURBT	None	2 days	N	P	P
AIIMS/JDH/2017/08/009457	56	M	TIG3	Urothelial	2.5	-	No	TURBT	None	2 days	N	P	N
AIIMS/JDH/2017/11/005522	55	M	TaG1	Urothelial	2.2	-	No	TURBT	None	3 days	N	P	N
AIIMS/JDH/2017/12/013408	45	M	T2	Urothelial	4.5	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2018/11/000305	49	M	T2	adenocarcinoma	1	-	No	random bladder biopsies	None	3 days	N	P	N
AIIMS/JDH/2018/09/004422Ghanshyam Barasa	67	M	T2	squamous	3.5	in bladder diverticulum	No	RC with IC	None	9 days	N	P	P
AIIMS/JDH/2018/09/006482	40	M	TIG3	sarcomatoid	3.8	-	No	TURBT	None	1 day	N	N	P
AIIMS/JDH/2016/06/008840	44	F	T2	squamous	9	multifocal	No	RC with IC	None	10 days	N	N	N
AIIMS/JDH/2018/03/007273Bhata Ram	67	M	T1G1	Urothelial	3.6	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2018/03/012681	58	F	TIG3	Urothelial	5.5	-	No	TURBT	None	1 day	N	P	P
AIIMS/JDH/2017/09/015038	66	M	T1G1	Urothelial	3.2	-	No	TURBT	None	2 days	N	P	N
AIIMS/JDH/2017/11/004110	69	M	TIG3	Urothelial	2.2	-	No	TURBT	None	2 days	N	P	N
AIIMS/JDH/2016/12/002686	59	M	T2	Urothelial	3.4	-	No	TURBT	None	1 day	N	P	N
AIIMS/JDH/2018/08/004355	67	M	TaG3	Urothelial	6	multifocal	No	TUR biopsy	None	1 day	N	N	P
AIIMS/JDH/2017/11/006537	48	M	T2	squamous	5.5	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2017/06/009577Karamai	49	F	T2	Urothelial	6	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2015/07/005150Badrinarayan Joshi	72	M	TaG3	Urothelial	1.5	-	No	TURBT	None	2 days	N	P	N
AIIMS/JDH/2018/10/002005	62	M	T1G1	Urothelial	3	-	No	TURBT	None	2 days	N	P	N
AIIMS/JDH/2021/12/011465	61	M	T1G1	Urothelial	3	multifocal	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2021/08/003650	32	F	TaG3	Urothelial	2.5	multifocal	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2021/09/012673	65	M	TaG1	Urothelial	2.5	-	No	TURBT	None	3 days	N	P	P
AIIMS/JDH/2021/12/002923	54	M	TaG1	Urothelial	1.5)	-	No	TURBT	None	3 days	N	P	P
AIIMS/JDH/2021/12/005785	61	M	TaG1	Urothelial	3	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2021/01/012172	65	M	T2	Urothelial	6	-	No	TURBT	cardiac arrest	Death	N	P	P
AIIMS/JDH/2020/09/009447	44	F	T1G1	Urothelial	9	-	No	TURBT	None	2 days	N	P	N
AIIMS/JDH/2015/08/006507	44	M	T2	Urothelial	5	-	No	TUR biopsy	NONE	2 DAYS	N	N	P
AIIMS/JDH/2016/06/000048	59	F	TaG1	Urothelial	4	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2018/08/008013	65	M	TaG1	Urothelial	1	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2021/08/001933	70	M	T2	Urothelial	5.4	-	No	TURBT	None	2 days	N	N	N
AIIMS/JDH/2019/06/000842	79	M	TaG1	Urothelial	2	-	No	TURBT	None	3 days	N	P	P
AIIMS/JDH/2018/03/016231	66	M	T1G3	Urothelial	3	-	No	TURBT	None	3 days	N	P	N
AIIMS/JDH/2019/06/011489	67	M	T1G3	Squamous	4.5	Multifocal	No	TUR biopsy	None	2 days	N	N	N
AIIMS/JDH/2019/07/007596	73	M	TaG1	Urothelial	1.3	-	No	TURBT	None	2 days	N	P	P
AIIMS/JDH/2019/10/002555	77	M	T1G3	Urothelial	4	-	No	TURBT	None	5 days	N	N	N
AIIMS/JDH/2018/07/005998	68	M	T3b	Urothelial	7.2	Multifocal	No	RC with IC	fever	Death	N	P	N
AIIMS/JDH/2019/11/017699	51	M	T2	Urothelial	7	-	No	TUR biopsy	None	1 day	N	N	P
AIIMS/JDH/2018/05/016706	62	M	TaG1	Urothelial	2.2	Multifocal	No	TURBT	Hematuria	4 days	N	P	P
AIIMS/JDH/2019/01/032091	66	M	TaG1	Urothelial	3	Multifocal	No	TURBT	iatrogenic urinary bladder perforation	4 days	N	P	N
AIIMS/JDH/2019/04/010187	49	M	T1G3	squamous	5.5	Multifocal	No	TURBT	None	4 days	N	N	N
AIIMS/JDH/2019/09/009220	58	M	TaG1	Urothelial	1.5	Multifocal	No	TURBT	None	1 day	N	N	P