अखिल भारतीय आयुर्विज्ञान संस्थान,जोधपुर
ALL INDIA INSTITUTE OF MEDICAL SCIENCES, JODHPUR
विकृति विज्ञान विभाग
DEPARTMENT OF PATHOLOGY & LAB. MEDICINE

संदर्भ :- एम्स/जेडीएच/ वि. वि /6315 /2023 दिनांक: 02/01/2023

सेवा में,

The Dean (Academics)
All India Institute of Medical Sciences
Jodhpur

Subject: Submission of M.D thesis.

आदरणीय महोदय,

This is to submit that the M.D thesis by the Academic Junior Resident (July 2020 Batch) of our Department have been duly completed and signed and is ready for submission. Please accept the same.

Details of her thesis are attached herein:

Name of candidate	Thesis topic
Dr. Apurva Arora	EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTOR 3 (FGFR3) & VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN MALIGNANT TUMORS OF THE UROTHELIAL TRACT

धन्यवाद,

भवदीया

02/01/2023

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EXPRESSION OF FIBROBLAST GROWTH FACTOR RECEPTOR 3 (FGFR3) & VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN MALIGNANT TUMORS OF THE UROTHELIAL TRACT

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfillment of the requirement for the degree of

DOCTOR OF MEDICINE (MD) PATHOLOGY

July 2020
AIIMS, JODHPUR

DR. APURVA ARORA

DECLARATION

I here declare that the thesis titled "**Expression of Fibroblast Growth Factor Receptor 3 (FGFR3) & Vascular Endothelial Growth Factor (VEGF) In Malignant Tumors of the Urothelial Tract**" embodies the original work carried out by the undersigned in All India Institute of Medical Science, Jodhpur

DR. APURVA ARORA
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CERTIFICATE

This is to certify that the thesis entitled **"Expression of Fibroblast Growth Factor Receptor 3 (FGFR3) & Vascular endothelial growth factor (VEGF) In Malignant Tumors of the Urothelial Tract"** is the bonafide work of **Dr. APURVA ARORA** carried out under our guidance & supervision, in the Department of Pathology & Lab Medicine, All India Institute of Medical Sciences, Jodhpur.

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Acknowledgement

Nothing worth achieving has ever been achieved without the guidance of our teachers and the support of family and friends. The immortal words of Helen Keller could not be more undeniable as I stand at the cusp of one of the most prestigious achievements of my life.

I would not be at this landmark moment without the trust, vision and patience of Dr. Aasma Nalwa, my MD thesis Guide, Additional Professor, Department of Pathology and Lab Medicine, All India institute of Medical Sciences, Jodhpur. I am truly honored and grateful for the opportunity to learn under her umbrella.

I am also indebted to all my Co-guides Dr. Poonam Abhay Elhence, Professor and Head, Department of Pathology and Lab Medicine, Dr. Meenakshi Rao, Additional Professor, Department of Pathology and Lab Medicine & Dr. Gautam Ram choudhary, Additional Professor, Department of Urology, All India Institute of Medical Sciences, Jodhpur. Their step by step support and guidance has been instrumental in creating the environment of success and excellence needed for a dissertation from our esteemed Institute.

Family is the backbone which has prevented me from ever faltering in the quest for excellence and knowledge. I cannot express in words my appreciation and love for my parents, Alpna & Harsh Arora, my sister Khyati and their immense support and understanding.

I acknowledge the nurturance I have received from seniors of the Department of Pathology and Lab Medicine, specially Dr. Rashim, & Dr. Vinay, Senior Residents. Without their help and reprimands, I would have been clueless as to how to proceed with the work involved in this dissertation. I also thank my juniors who have stepped up in times of need to help balance the duties of a junior resident of our beloved Department and the commitments to my thesis.

All the faculties and my co-supervisors of the Department of Pathology and Lab Medicine, All India Institute of Medical Sciences, Jodhpur have been pillars of support and understanding and this would not have been possible without them. I also hope I have been as good a friend and co-PG to Dr. Sangeeta, Dr Deepshikha & Dr. Tanya as they have been to me.

I would like to specially acknowledge Dr. Akhil Goel, Assistant professor, Department of Community Medicine and Family Medicine for teaching me how to statistically analyze the

data presented in this thesis.

Last, but not the least, I express my thanks to all the laboratory technicians of our department for always being ready to help. I would like to specially mention Mrs. Meenakshi ji, for without her technique and practical application of sectioning of tissue till Immunohistochemistry application would not have been possible.

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SYNOPSIS

In the present study, we assessed the **Expression of Fibroblast Growth Factor Receptor 3 (FGFR3) & Vascular Endothelial Growth Factor (VEGF) In Malignant Tumors of the Urothelial Tract** by immunohistochemistry and correlated its expression with clinicopathological features. The IHC marker FGFR3 & VEGF were applied manually in all 79 cases of urothelial carcinoma & the scoring was done using a semi-quantitative (Q-score) scoring system.

The present study was undertaken in a tertiary care hospital of western Rajasthan. A total of 79 cases of malignant tumors of the urothelial tract (n=79) were included in the study. Of these, 52 (65.8%) cases showed FGFR3 positivity & among these 32 (61.5%) cases were HGUC, which included 25 (78.1%) non-muscle invasive HGUC & 7 (21.9%) deep muscle invasive HGUC. 19 (36.6%) cases of LGUC showed FGFR3 positivity. Of these, all were non-muscle invasive LGUC. No deep muscle invasive FGFR3 positive LGUC was seen. 1 (1.9%) case of squamous cell neoplasm of the urinary tract also showed FGFR3 positivity.

Out of total 79 cases, 64 (81.01%) cases showed VEGF positivity & among these 39 (60.9%) cases were HGUC, which included 10 (25.6%) non-invasive HGUC, 20 (51.3%) lamina propria invasive HGUC & 9 (23.1%) deep muscle invasive HGUC. VEGF positive 22 (34.4%) cases of LGUC included 16 (72.7%) non-invasive LGUC & 6 (27.3%) cases of lamina propria invasive LGUC. No deep muscle invasive VEGF positive LGUC were seen.

It was observed that positive expression of FGFR3 & VEGF [45 (86.5%) cases & 55 (85.9%) cases respectively] was significantly high in non-muscle invasive urothelial carcinomas. However, no positive expression was seen in Low grade muscle invasive urothelial cancer & among high grade muscle invasive urothelial carcinoma, positive expression of FGFR3 & VEGF was shown by 7 (21.9%) & 9 (23.1%) cases, respectively.

LIST OF ABBREVIATIONS

AJCC	The American Joint Committee on Cancer
ARB	Antigen Retrieval Buffer
ASCOCAP	American Society of Clinical Oncology, College of American Pathologists
BCG	Bacillus Calmette-Guerin
CIS	Carcinoma in-situ
DAB	Diaminobenzidinetetrahydrochloride
DAPI	Diamidino-2-phenylindole-2 Hydrochloride
D	Deep muscle invasive
EDTA	Ethylenediaminetetraaceticacid
EGFR	Epidermal growth factor receptor
FGFR	Fibroblast growth factor receptor
FR	Forerunner
FISH	Fluorescent in-situ hybridization
GLOBOCAN	Global Cancer Incidence, Mortality &Prevalence
H&E	Haematoxylin & eosin
HG	High grade
HGUC	High Grade Urothelial Carcinoma
HGIN	High-grade intraepithelial neoplasia
HRP	Horse radish peroxidase
HCl	Hydrochloric acid
IARC	International Agency for Research on Cancer
IHC	Immunohistochemistry
I	Invasive
ISUP	The International Society of Urological Pathology
LG	Low grade
LOH	Loss of heterozygosity

LGIN	Low-grade intraepithelial neoplasia
LVI	Lymphovascular Invasion
MAPK	Mitogen-activated protein kinase
MIBC	Muscle-invasive bladder cancer
N:C	Nucleo-cytoplasmic
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NEC	Neuroendocrine carcinoma
NMIBC	Non-Muscle invasive bladder cancer
PI3K	Phosphatidylinositol-4,5-bisphosphate3-kinase
PKC	Protein kinase C
PLL	Poly-L-Lysine
PNI	Perineural invasion
PUNLMP	Papillary urothelial neoplasm of low malignant potential
RCP	Radical Cystoprostetectomy
SCC	Squamous cell carcinoma
SC-NEC	Small cell Neuroendocrine carcinoma
SSC	Saline sodium citrate buffer
SPSS	Statistical package for the social sciences
TNM	Tumor-Node-Metastasis
TURBT	Transurethral resection of bladder tumor
UBC	Urinary bladder cancer
VEGF	Vascular endothelial growth factor
V/V	Volume per volume
WHO	World Health Organization

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INTRODUCTION

Urothelial carcinoma is the ninth most common malignancy in the world & most common malignancy of the urinary tract(1,2). It accounts for approximately 3% of the global cancer burden, according to the latest GLOBOCAN data(2). It is more common in males than females. Urothelial carcinoma accounts for approximately 5.3% of all the genitourinary cancers in Indian males(3).

The tumors of urinary tract include: Urothelial tumors, also known as transitional cell carcinoma, & its subtypes, squamous cell neoplasms, glandular neoplasms & urethral neoplasms. Urothelial carcinomas harbor a large proportion of recurrently mutated genes in key pro-tumorigenic signaling pathways, & studies have investigated a number of potential molecular targets such as mTOR & Her2(4).

Fibroblast growth factor receptor (FGFR3) belongs to the tyrosine kinase family & regulates various cellular functions including angiogenesis, differentiation, cell survival & carcinogenesis by regulating the downstream pathways including RAS-MAPK, STAT6 & P13K(5). FGFR3 mutations have been found in around 80% of tumors stage pTa, 21% of pT1, & 16% of pT2-4 urothelial tumors(6). Among these mutations, most common type are the activating mutations followed by gene rearrangements & amplification(5).

Angiogenesis is the process of formation of new blood vessels for the delivery of oxygen & the nutrients for the development & repair of the tissues by activation of the endothelial cells (4). Malignant tissues are stressors that activate the endothelial cells to maintain vascular framework, nutrient & oxygen supply. Vascular Endothelial Growth Factor (VEGF) is an important angiogenic agent which was first demonstrated by Chodak et al as an important proangiogenic agent in patients with transitional cell carcinoma(7). VEGF-A interacts with the VEGFR2 to regulate the proliferation & migration of endothelial cells, causing vascular proliferation & maintaining neovascularization(8).

The prognostic factors in various subtypes of bladder carcinoma are depth of invasion, histological grade, margin status, angiogenesis, in which VEGF is the major factor, p53 expression, Ki-67, loss of E-cadherin, CK20 & FGFR3 mutations(6, 9).

Targeted treatment of urothelial carcinomas with FGFR3 mutations is in various stages of trials along with the emerging concept that as an alternative to targeting intrinsic tumor growth pathways, targeted therapies can be used to modulate the tumor vasculature with the

aim of improving the tumor uptake of drugs such as chemotherapy drugs & VEGF receptor2 targeted therapy has shown encouraging results in patients with urothelial carcinomas.

REVIEW OF LITERATURE

- **Embryology**

The formation of urogenital ridge marks the beginning of development of the urinary tract. The urinary bladder develops from a hindgut structure, the cloaca, that acts as a common chamber for gastrointestinal & urinary tract. The urorectal septum divides the cloaca dorsally into rectum & ventrally into urogenital sinus in-between the 4th to 7th week of gestation. Majority of the urinary bladder develops from urogenital sinus. The bladder trigone is formed by the fusion of the caudal portion of mesonephric ducts with urogenital sinus in the midline. Ureteric bud, a protrusion from the mesonephric duct, gives rise to the ureter which opens in to the bladder at the area of the trigone(9). The epithelium of the urinary bladder is endodermal in origin, derived from the cranial portion of the urogenital sinus. The unaltered embryonic epithelium that varies from columnar to low cuboidal, gets converted to multi-layered epithelium at around 10th week of gestation. The adjacent splanchnic mesenchyme gives rise to the lamina propria, the muscularis propria & the adventitia(9).

- **Anatomy**

The bladder is a subperitoneal, hollow viscus divided into upper & lower part, with upper part comprising of apex & body while fundus, trigone & neck constitute the lower part. Its shape is of a four-sided inverted pyramid, when empty & of a rounded structure, when distended. The trigone, located at the base of the bladder, is in continuation with the bladder neck, in which posterior & infero-lateral walls converge to open into the urethra(9). The transverse section of the bladder shows mucosa, muscularis propria, & adventitia. In a relaxed urinary bladder, the urothelium is five to seven layers thick while in the distended bladder, the urothelium reorganizes to two or three layers without any structural damage. Due to this transitional ability of the urothelium, it is also known as the transitional epithelium(10). The apical layer is a single layer of umbrella-shaped cells that are frequently binucleated. The intermediate layer is formed of two to three layers of polygonal cells. The basal layer is formed from two to three layers of small cuboidal cells(10).

- **Etiological factors & gender distribution**

Risk factors for urothelial carcinoma can be divided into modifiable & non-modifiable factors. Modifiable factors include smoking, occupational exposure to aromatic amines, dietary supplements containing aristolochic acid etc, which can be avoided to prevent development of cancer. Non-modifiable factors like age, gender, chronic infection, genetic & family history including Cowden syndrome & Lynch syndrome, make the person more susceptible for cancer development(11).

- **Clinical features-signs & symptoms (WHO)**(12)

Clinical presentation & severity of the symptoms depends on the site & extent of the tumor. Most common symptom is hematuria, microscopic or gross. Large bladder tumors can lead to increased frequency while those located in the neck may present with irritative symptoms i.e. dysuria, urgency & frequency. Patient may present with hydronephrosis, in case of tumor infiltrating the ureteral orifice. Suspected cases are usually subjected to cystoscopy. Other investigations like transabdominal ultrasound & computed tomography urography can be done.

WHO CLASSIFICATION OF TUMORS OF UROTHELIAL TRACT (5TH EDITION) (13)

➢ ***Urothelial tumors***

Invasive urothelial neoplasms

- Invasive urothelial carcinoma
- Conventional urothelial carcinoma
- Nested
- Large nested
- Tubular
- Microcystic
- Micropapillary
- Lymphoepithelioma-like
- Plasmacytoid
- Sarcomatoid
- Giant cell
- Poorly differentiated

- Lipid-rich
- Clear cell

• Non-invasive urothelial neoplasms

- Urothelial papilloma
- Urothelial papilloma, Inverted
- Papillary urothelial neoplasm of low malignant potential
- Non-invasive papillary urothelial carcinoma, low-grade
- Non-invasive papillary urothelial carcinoma, high-grade
- Urothelial carcinoma in situ

➢ ***Squamous cell neoplasmof the urinary tract***

• Pure squamous cell carcinoma of the urothelial tract
• Verrucous carcinoma
• Squamous papilloma

➢ ***Glandular neoplasms***

• Adenoma

- Villous adenoma
- Tubular adenoma
- Tubulo-villous adenoma

• Adenocarcinoma

- Adenocarcinoma, NOS
- Enteric adenocarcinoma
- Mucinous adenocarcinoma
- Mixed adenocarcinoma
- Signet ring cell adenocarcinoma
- Adenocarcinoma in-situ

➢ ***Urachal carcinoma& diverticular neoplasms***

• Urachal carcinoma
• Invasive urothelial carcinoma

➢ ***Tumors of Mullerian type***

• Clear cell carcinoma
• Endometrioid carcinoma

➢ ***Urethral neoplasms***

➢ ***Miscellaneous tumors***

- Carcinoma of Skene, Cowper, & Littre glands
- Metastatic tumors & tumors extending from other organs
- Epithelial tumors of the upper urinary tract
- Tumors arising in a bladder diverticulum
- Urothelial tumors of the urethra

TUMOR STAGING

Currently, the most recent staging system is the one developed jointly by The American Joint Committee on Cancer (AJCC 8th edition) also termed Tumor-Node-Metastasis (TNM) (14)

This staging system enables the simultaneous description of primary tumor extent (T), status of lymph nodes (N), & the extent of distant metastasis (M).

TNM CLASSIFICATION OF URINARY BLADDER CARCINOMA(14)

TX Primary tumor could not be assessed

T0 No evidence of primary tumor

Ta Non-invasive papillary carcinoma

Tis Carcinoma in-situ "flat tumor"

T1 Tumor invades subepithelial connective tissue

T2 Tumor invades muscularis propria

T2a Tumor invades superficial muscularis propria (inner half)

T2b Tumor invades deep muscularis propria (outer half)

T3 Tumor invades perivesical tissue

T3a Microscopically

T3b Macroscopically (extravesical fat)

T4 Tumor invades any of the following: prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall

T4a Tumor invades the prostatic stroma, seminal vesicles, uterus, or vagina

T4b Tumor invades the pelvic wall or abdominal wall

NX Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Metastasis to a single lymph node in the true pelvis (perivesical, obturator, external & internal iliac or sacral lymph node)

N2 Metastasis to multiple regional lymph nodes in the true pelvis (hypogastric, obturator, external & internal iliac or sacral lymph nodes)

N3 Metastasis to common iliac lymph nodes

M0 No distant metastasis

M1 Distant metastasis

M1a Distant metastasis limited to lymph nodes beyond the common iliac

M1b Non lymph node distant metastasis

AJCC PROGNOSTIC STAGE GROUPS (14)

STAGE 0a	Ta	N0	M0
STAGE 0is	Tis	N0	M0
STAGE I	T1	N0	M0
STAGE II	T2a	N0	M0
STAGE II	T2b	N0	M0
STAGE III A	T3a, T3b, T4a	N0	M0
STAGE III A	T1-T4a	N1	M0
STAGE III B	T1-T4a	N2,N3	M0
STAGE IV A	T4b	Any N	M0
STAGE IV A	Any T	Any N	M1a
STAGE IV B	Any T	Any N	M1b

Pathogenesis of urothelial carcinoma(16, 17)

Two distinct molecular pathways have been identified by the analysis of genomes of precursor lesions & invasive urothelial carcinomas

1. One pathway exhibit gain of function mutations, including amplification of the FGFR3 tyrosine kinase receptor gene or activating mutations in the genes encoding RAS & PI 3-kinase, leading to increase signaling through growth factor receptor pathway. This alteration is most commonly seen in "non-muscle invasive papillary carcinomas"
2. Muscle invasive bladder carcinomas develop most commonly through the progression from 'flat' carcinoma in situ, having mutations that disrupt the function of p53 & RB gene

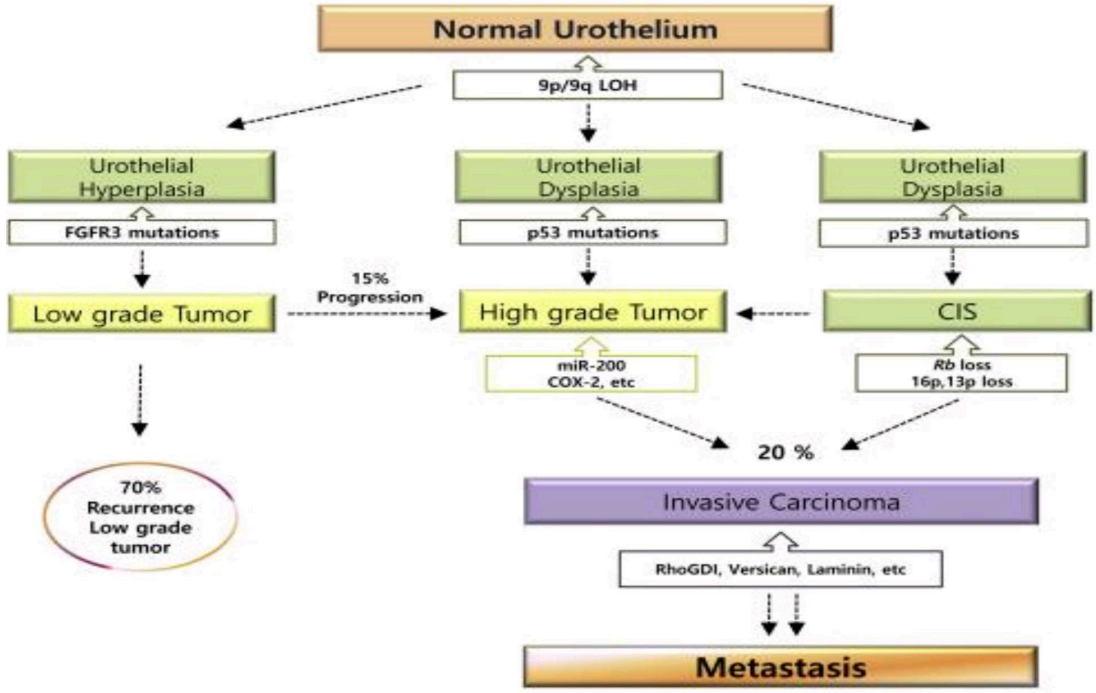


FIGURE 1:

Putative molecular pathways of oncogenesis in low & high grade urothelial carcinoma of the urinary bladder(15) (reproduced from Shin, J. H., et al (2018). Pathophysiology of Bladder Cancer. Bladder Cancer, 33–41. doi:10.1016/b978-0-12-809939-1.00003-5)

Fibroblast growth factor receptor, FGFR3

Fibroblast growth factor receptor belongs to a family of transmembrane tyrosine kinase receptors & constitutes four different receptors (FGFR1-FGFR4). Located in the cell membrane, these are formed by extracellular, transmembrane & intracellular domains(16). These receptors mediate numerous physiological processes, including proliferation, differentiation, migration & apoptosis, as they exhibit autophosphorylation activity(17). Also its structural activation is closely related to many diseases, including cancer.

FGFR3 protein encoded by FGFR3 gene, located on chromosome 4, is expressed by chondrocytes & osteoblasts & thus plays an important role in osteogenesis & bone maintenance. FGFR3 gene alterations are implicated in bone growth disorders & wide range of cancers, with prevalence of FGFR3 gene aberrations being highest in urothelial carcinomas, followed by uterine carcinosarcoma, esophageal, ovarian & endometrial cancers(18).

FGFR3 signaling pathway alterations, most commonly involving RAS-MAPK pathways, are found more commonly in bladder carcinomas with aberrant activation of the receptor occurring via various mechanisms, including FGFR3 point mutation & upregulated expression & isoform switching(16). FGFR3 mutations, activation & overexpression have different effects on downstream signaling & phenotypic consequences & is related to tumor stage & grade (17),(19). In low-grade bladder cancer, the rate of FGFR3 mutation is high (approximately 80%), resulting mainly due to FGFR3 overexpression(17). Recent studies showed a higher rate of FGFR3 gene mutations in the upper tract (16).

Studies based on mutational & expression data suggest that >80% of non-muscle-invasive bladder cancers (NMIBC) & approximately 40% of muscle-invasive bladder cancers (MIBC) reveal up-regulation of FGFR3 signaling(19). FGFR3 gene alteration is associated with lower grade & stage of urothelial carcinoma & hence constitutes as one of the prognostic indicators. Therefore, FGFR3 targeting therapies are now being developed for urothelial carcinomas exhibiting FGFR3 as an oncogenic driver & are at various phases of trials(18).

Vascular endothelial growth factor, VEGF

Angiogenesis involving the proliferation of endothelial cells is an essential physiological process for normal development & tissue repair. Normally this phenomenon is regulated by various mechanisms, thus maintaining the non-angiogenic phenotype. Transformation of endothelial cells to an angiogenic phenotype is triggered by numerous stressors such as tissue growth, inflammation, immune cell activation & hypoxia leading to neovascularization(20). In order to maintain adequate nutrient & oxygen supply, malignant tissue also induces this switch of endothelial cell transformation, as tumor progression along with metastasis is angiogenesis dependent(21).

VEGF is a powerful angiogenic agent in comparison to other angiogenic factors, like basic fibroblast growth factor (bFGF), angiogenin, transforming growth factor (TGF)-α, TGF-β etc (21). It also induces endothelial cell permeability, causing extravasation of plasma protein further resulting in growth of new blood vessels(22). VEFG-A, one of the glycoproteins in the VEGF family acts selectively on vascular endothelial cells & causes stimulation of angiogenesis in vitro & in vivo, is produced by different cell types in the body including epithelial cells, inflammatory & hematopoietic cells, endothelial cells(21). Therefore, VEGF-A is now being targeted for anti-angiogenic therapy.

Evidence also exists to support the involvement of VEGF in several cancer types, including bladder carcinoma. In last several years, its expression in various tumors, like Kaposi sarcoma, melanoma, ovarian carcinoma, squamous cell carcinoma of the head & neck & breast carcinoma has been reported & anti-angiogenic drugs, primarily Bevacizumab, Sorafenib & Sunitinib have already been approved for use in many advanced tumors, as they are seen to significantly improve the treatment of cancer(23).

1. Laura S Mertens et al in 2022 (24) carried out a multicentric, multi-laboratoy analysis in 1058 radical cystectomy patients to view FGFR3 mutation status versus p53 & Ki-67 expression. The study showed FGFR3 mutation in 107 (10%) cases which was associated with lower pT stage, grade & pNo. Aberrant p53 expression was observed in 718 (68%) tumors while 55% i.e. 581 cases showed aberrant Ki-67 expression & were associated with adverse tumor characteristics. Hence, they concluded that to guide adjuvant treatment & follow-up strategies, FGFR3 mutation might represent a valuable tool.

2. Anika Sadaf et al in 2021(23) did a cross-sectional study & established a significant association of VEGF with tumor grade, along with its inverse association with muscle invasion. They analyzed 56 cases of bladder carcinoma, from TURBT samples & observed weak to strong positive expression of VEGF in all high grade carcinomas (31 cases) while all NMIBC cases were positive for VEGF expression with 55.56% (5 cases) showing strong positivity. Concluding that expression of VEGF was higher among high grade & non-muscle invasive bladder carcinoma.

3. Rhijn et al (25) in 2020 assessed the prognostic value of FGFR3 & p53 in 1000chemotherapy-naïve radical cystectomy specimens & analyzed FGFR3 mutations, FGFR3 & p53 protein expression. According to the study FGFR3 overexpression was found in 28% & p53 overexpression was found in 69% of tumors. In their study, they also observed that FGFR3 overexpression was associated with lower pT stage & tumor grade.

4. Alec Kacew & Randy F. Sweis(18) in 2020 reviewed the role of FGFR3 as a prognostic & predictive marker in urothelial bladder carcinoma & concluded that FGFR3 gene alterations were associated with lower grade, stage & had clinically less aggressive behavior. According to their study, 49-84% of non-muscle invasive cases & only 18% of muscle invasive cases expressed FGFR3 & FGFR3 protein targeted therapies had clinically benefitted some patients.

5. Malik et al(6) in 2019 retrospectively analyzed 55 urothelial carcinoma specimens. Out of which 66.7% of the high grade non-invasive urothelial & 82.6% of the low-grade non-invasive urothelial carcinomas showed immunohistochemical expression of

FGFR3 while only 18% cases of the high-grade invasive carcinoma showed FGFR3 expression. FGFR3 was expressed in 14.3 % of high-grade invasive tumors which recurred. High grade non-invasive tumors were positive for FGFR3 in 80% of the cases.

6. In 2019, Nassar et al(26) analyzed the mutation spectra by using targeted exome sequencing, in 82 low-grade non muscle-invasive bladder cancers (LG-NMIBC), 199 muscle-invasive bladder cancers (MIBC), 126 high-grade (HG) NMIBC, including 10 LG-upper tract urothelial cancers (LG-UTUC), & 55 HG-UTUC. According to the study they observed that FGFR3 & KDM6A mutations were significantly more common in LG-NMIBC (72% & 44%, respectively) as compared to other bladder subtypes. It was also observed that FGFR3 alterations were enriched in LG-UTUC than HG-UTUC tumors (80% vs.16%).

7. Kim et al(5) in 2018 analyzed radical cystectomy & ureteronephrectomy specimens from 74 urothelial carcinoma patients. Among these, 16 (22%) patients harbored FGFR3 alterations & the frequency of FGFR3 aberrations was found to be higher in bladder urothelial carcinomas (25%) than in urothelial carcinoma of the renal pelvis & ureter (18%).

8. Behl et al(28) in 2017 studied the expression of VEGF in 50 cases of urothelial carcinoma & concluded that VEGF expression was higher in patients with high grade tumor as compared to low grade tumor.

9. In 2016, Lukasz Piotr Fus & Barbara Gornicka (29) studied the proangiogenic factors expression, including VEGF, HIF-1, bFGF, IL-8 & MMPs, in bladder tumors. According to their study, disease progression & shorter survival was correlated with high expression of pro-angiogenic factors.

10. In 2016 D Pouessel et al, by using PCR-SNaPsot method, evaluated 61 TUR & 614 radical cystectomy specimens for FGFR3 heterogeneity. Their study showed that among TUR samples, 13/34 (38%) T1 & 8/27 (30%) $\geq$ T2stage tumor harbored FGFR3 mutations. Within RC specimens, FGFR3 mutation was found in 11% (67/614) cases.

11. Arshad Rahmani et al(30), in 2012, analyzed a total of 125 cases of histopathologically confirmed Transitional cell carcinoma (TCC) along with 100 cases of confirmed inflammatory lesions of urinary bladder as control. They reported VEGF expression to be more prevalent in advanced & progressing bladder carcinoma, thus indicating a strong positive correlation between VEGF expressions & tumor grade (Grade I- 36.8%, Grade II - 44.6% & Grade III - 47.5%).

12. 55 cases of primary bladder cancers were examined by Young-Heemaeng, Su-yongEnu & Jung-Sik Huh in 2010(31), in which they observed that cytoplasmic FGFR3 positivity on IHC, was associated with not only with lower grade & stage but also in predicting the disease recurrence. As according to their study, it was observed that patients with higher stage, negative FGFR3 cytoplasmic staining & high Ki-67 had recurrence more frequently.

13. In 2008, DC Tomlinson et al (32) by using direct sequencing, screened 158 urothelial carcinoma samples for mutations in FGFR3 exons & its association with tumor grade and stage. They also examined the FGFR3 expression by IHC & its correlation with tumor stage, grade & mutation status. The IHC was carried out on 149 samples & they observed that expression of FGFR3 was significantly higher in non-invasive (pTa), as compared to invasive tumors (pT2). They also observed that the FGFR3 expression was associated with low grade as compared to high grade urothelial carcinoma. According to their study, 85% of mutant tumors showed over-expression & among them most were low grade (74% of mutant high expressers were grade 1 or 2) or stage (73% non-invasive pTa). 42% of wildtype tumors showed overexpression, 66% were high grade & 68% were invasive (pT1 or pT2)

14. In 2005, J Javier Gomez-Roman et al (27) observed the overexpression of FGFR3 in urinary tract carcinomas by using the microarray tools, western blotting & IHC. They found FGFR3 mRNA overexpression in pTa & pT1 stage carcinomas (fold change >8) & in pT2 carcinomas, fold change >4. Similarly, on western blotting 83% of pTa, 100% of pT1 & 50% of pT2 carcinomas showed FGFR3 expression. 71.4% of pTa, 72% of pT1 & 49.2% of pT2 expressed FGFR3 by IHC.

15. In 2004, Ching-Chiang Yang , Kang-Chu Chu, Wen-Meng Yeh (33) aimed to investigative & correlate the expression of the VEGF gene & its clinical significance in transitional cell carcinoma (TCC) of urinary bladder. They studied the cohort of 161 patients with TCC, with an immunohistochemical stain for the expression of the VEGF gene. They concluded that there was significant increase in positive rate of VEGF gene expression with the progression of tumor grade & clinical staging. It was also revealed VEGF gene expression was proportional to the formation & progression of TCC.

AIMS & OBJECTIVES

AIM

To assess the expression of Fibroblast Growth Factor Receptor 3 (FGFR3) & Vascular Endothelial Growth Factor (VEGF) in malignancy of urothelial tract.

OBJECTIVE

- Primary objective:
- To assess the Fibroblast Growth Factor Receptor 3(FGFR3) expression in urothelial tract malignancies by immunohistochemical evaluation.
- To assess the Vascular Endothelial Growth Factor (VEGF) expression in urothelial tract malignancies by immunohistochemical evaluation.

- Secondary objective:
- To correlate the FGFR3 expression with clinicopathologic parameters like histologic subtype, tumor stage, grade & lymph node involvement in all the cases of urothelial tract malignancies.
- To correlate VEGF expression with clinicopathologic parameters like histologic subtype, tumor stage, grade & lymph node involvement in all the cases of urothelial tract malignancies.
- To assess the correlation between FGFR3 & VEGF expression with clinicopathologic parameters like histologic subtype, tumor stage, grade & lymph node involvement in all the cases of urothelial tract malignancies.

MATERIAL & METHODS

Study Design: Ambispective type of observational study

Study setting: Department of Pathology & Lab Medicine & Department of Urology, All India Institute of Medical Sciences, Jodhpur.

Source of data: This study included urothelial carcinomas diagnosed on samples received as trans-urethral resection of bladder tumor (TURBT), cystectomy & cysto-nephrectomy specimens. All the urothelial carcinomas including muscle invasive & non-muscle invasive, both low grade & high grade, received in the Department of Pathology & Lab Medicine at AIIMS Jodhpur from July 2016 to July 2022 were included in the study. Haematoxylin & Eosin-stained slides of the diagnosed cases were retrieved from the departmental archives. Approval from an institutional review board was obtained at the initiation of the study.

Ethical clearance: The study was approved by the institutional ethical committee on 12 March 2021, bearing Certificate No: AIIMS/IEC/2021/3510 (Appendix-1)

Study variables:

- Microscopic features of bladder tumor, grade, stage, lymphovascular invasion, perineural invasion, any other differentiation
- Immunohistochemistry for FGFR3 & VEGF on paraffin-embedded tissue blocks in all urothelial carcinoma cases
- Correlation of FGFR3 & VEGF status with the clinic-pathological features like age, gender, histological grade, tumor stage, subtypes of urothelial carcinoma differentiation like squamous/sarcomatous, lymphovascular invasion & perineural invasion

INCLUSION CRITERIA:

- All the malignancies of the upper & lower urothelial tract as per WHO 2022 classification of the tumors of the urothelial tract, who did not receive chemotherapy

EXCLUSION CRITERIA:

- All bladder tumor samples of patients who received chemotherapy
- Invasion & metastasis of tumors other than urothelial carcinoma to the bladder
- Inadequate samples

- Any previous cancer for which chemotherapy or radiotherapy was given

Statistical analysis: Data was entered in the excel sheet & analyzed by IBM-SPSS software 23.0 version. For correlation, MannWhitney U test was used & for survival, Kaplan-Meier was used.

A total of 79 cases were included in the study. Quantitative data like FGFR3 & VEGF expression was considered.

METHOD OF DATA COLLECTION:

- ➢ History, radiology & other investigations
- ➢ Cystectomy specimen or TUR of bladder tumor
- ➢ Light microscopic findings of bladder tumor by H&E staining
- ➢ IHC staining for FGFR3 & VEGF

EXPERIMENT DESIGN: Descriptive, Observational, Cross-sectional study

SAMPLE PROCESSING, STAINING & IMMUNOHISTOCHEMISTRY

The study was started after obtaining the approval from institutional review board. Informed consent was obtained from the patients. Specimens were received from the Department of Urology, AIIMS, Jodhpur & received in the Department of Pathology & Lab Medicine at AIIMS, Jodhpur along with duly filled up consent & case record forms. After receiving, the biopsies were measured, described & put into cassettes. The cystectomy & TURBT specimens were examined grossly. The gross descriptions such as size, weight, color, cut surface, consistency, areas of haemorrhage, & necrosis were described.

Grossing of radical cystectomy specimen(34)

The specimen was oriented & attached relations with adjacent attached organs like urethra were discerned. In males, prostate, vas deferens, seminal vesicle were mentioned separately. The size of the bladder, ureteric stumps & urethra was documented. The bladder was probed through the urethral orifice & cut open anteriorly to expose the tumor. The ureters were opened from the point of their resection margin up to their opening in the bladder. The tumor was then examined concerning size, location, presence of multifocal tumors, cut surface, depth of invasion to the bladder wall & extension into the perivesical tissue. Relevant sections were taken including margins, urethral cut margins, right & left ureteric cut margin,

vas deferens cut margin in males. Sections from the tumor including full-thickness of the tumor, areas with tumor infiltration into the bladder wall & tumor along with perivesical fat, & inked resection margin were taken. Other sections included ureteric orifices, bladder neck, trigone, anterior & posterior wall & dome of the bladder. The prostate was sectioned, beginning at the bladder neck & extending across through the urethra to the distal cut margin & prostate along with seminal vesicle was submitted in entirety. In the case of females, sections from the attached specimen of the uterus with cervix were also given.

Paraffin-embedded tissue blocks were prepared using routine histopathological techniques. Thin sections (3 µm) were stained with routine Haematoxylin & Eosin stain. Light microscopy results were recorded & histopathological grading as per the WHO/ISUP 2016 classification was given. An appropriate representative block was subjected to IHC for FGFR3 & VEGF. As per "The Human Protein Atlas", cytoplasmic & membranous immunohistochemical expression of FGFR3 & VEGF was considered positive. IHC parameters for FGFR3& VEGF were assessed as described (35,36).

1) STEPS OF BLOCK PREPARATION & SECTION CUTTING

After the relevant sectioning the tissue was processed as follows:

1. Dehydration was carried out by passing the sections through a series of ascending grades of ethyl alcohol, from 50%, 70%, 95% to absolute alcohol
2. The clearing was done by passing the tissue through two changes of xylene
3. Impregnation was done in molten paraffin wax which had a melting point of 54 – 62˚C
4. Embedding was done using Embedding station (Leica EG 1150 H) through which a small amount of liquid paraffin was layered into aluminium molds. Properly oriented tissues were placed inside the molds, which were then filled with liquid paraffin (60 – 62˚C) & allowed to cool & harden. The lower portion of the cassette with an identification number was used as the final block.
5. Microtomy: Microtome (Leica-RM2255) was used & thin ribbons (3µm) were cut & floated in warm water (~56˚C) for expansion of the curled

sections. These sections were then collected on frosted glass slides & kept for drying.

2) STAINING OF SECTIONS: (For H & E Stain)

1. De-paraffinization–The glass slides containing the tissue sections were kept over the hot plate at 60˚C for 10 minutes, followed by two changes in Xylene (Xylene I & Xylene II), 10 minutes each.
2. Hydration – Through graded alcohol (100%, 95%, 70%, 50%) to water, 3 minutes each.
3. Haematoxylin–The sections were kept in Harris Haematoxylin for 5-10minutes.
4. Washing–The sections were washed well in water for 2minutes.
5. Differentiation was done in 1% acid alcohol (1% HCl in 70% alcohol) for 10 seconds.
6. Washing–Done under running tap water (usually for 3-5 minutes) until the sections 'blue'.
7. Eosin– Stained in 1%Eosin Y for 5-6seconds
8. Washing–Done in running tap water for 2 minutes.
9. Dehydration–Through graded alcohol (50%, 70%, 95%, 100%) 2 minutes each.
10. Clearing–Through xylene (Xylene II & Xylene I), 2 minutes each.
11. Mounting–The sections were mounted using automated cover slipper (Leica C5030).

Immunohistochemistry (37)

The immune system exerts its control through humoral & cellular components. When lymphocytes are exposed to antigens, some of them proliferate. Each lymphocyte forms a clone of cells & all cells in a single clone produce an identical antibody. But various clones produce antibodies of different classes with specificities to different molecular sites on the antigen. Antigenic stimulation produces a mixture of antibodies from many of these clones of lymphocytes. This pool of antibodies is known as a polyclonal antibody. Monoclonal antibodies are prepared by injecting mice with an antigen. B-lymphocytes harvested from the mouse spleen are fused with non-secretory myeloma cells. This in-vitro fusion yields hybrid cells (hybridoma) that can be cloned. A single clone is capable of producing the antibody, having an identical molecular structure in unlimited quantities. They can be characterized, standardized & produced in unlimited quantities. Horse radish peroxidase (HRP) is the enzyme most commonly chosen for coupling to antibody. HRP, in presence of hydrogen peroxide & a chromogen (3,3-diamino benzidine tetrahydro chloride -DAB), will identify the site of antibody binding by forming a crisp, insoluble, stable, dark-brown colored reaction end product. The polymer –HRP detection system is an over detection system which uses an on-biotinpolymeric technology. Therefore, the problems associated with biotin, like non-specific nuclear staining, non-specific background staining, are eliminated.

ANTIBODIES USED:

1. **Primary Antibody**:

   - FGFR3: Concentrated FGFR-3 (B-9), a mouse monoclonal antibody raised against amino acids 25-124 of FGFR-3 of human origin, SANTA CRUZ BIOTECHNOLOGY, INC, was used in1:50 dilution at 9 pH.

   - VEGF: Concentrated VEGF (C-1): sc-7269, mouse monoclonal antibody raised against amino acids 1-140 of VEGF of human origin, SANTA CRUZ BIOTECHNOLOGY, INC, was used in 1:50 dilution at 9 pH.

2. **Secondary Antibody:** BOND Polymer Refine Detection (Leica Biosystems, New castle, UK)

- Peroxide block, 3-4% (v/v-volume per volume)
- Post Primary, Rabbit anti-mouse IgG in10% (v/v) animal serum in tris-buffered saline

- Polymer, Anti-rabbit Poly-HRP-IgG containing 10% (v/v) animal serum in tris-buffered saline
- DAB Part 1,in stabilizer solution
- DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
- DAB Part B ≤0.1% (V/V) Hydrogen peroxide in stabilizer solution
- Haematoxylin, 0.1%

STEPS OF IHC STAINING

A. Preparation of Buffer–Two types of buffers were used

- Wash Buffer
- Antigen Retrieval Buffer (ARB)
- Wash buffer preparation: 6 gm powdered TRIS buffer salt was dissolved into 1 liter of distilled water & pH was set at 7.4.
- ARB preparation: 6.05gm TRIS salt & 0.744gm EDTA salt were dissolved in 1 liter of distilled water, pH was set at 9.0

NOTE:

- To increase the pH, NaOH solution was added drop by drop & pH was titrated
- To decrease the pH, HCl was added drop by drop & pH was titrated

B. Preparation of Poly-L-Lysine Solution (PLL Solution):

-1ml of PLL was diluted with 9 ml of distilled water (1 in 10 dilutions)

C. Slide Coating Procedure:

Step1: Diluted PLL solution was taken in a clean container/Coplin jar

Step2: Both sides of glass slides were cleaned with tissue paper

Step 3: The clean slides were immersed in a PLL solution for 5 minutes

Step 4: After 5 minutes, the coated slides were removed & kept overnight for air drying. The coated slides were kept at room temperature. Tissue sections of 4µ thickness were obtained on the PLL coated slides

Baking: The slides were kept at 60˚C for 1 hour & then cooled to room temperature

IHC STAINING PROCEDURE

Step 1: Deparaffinization – The slides were kept in Xylene I (10 minutes), followed by Xylene II (10 minutes)

Step 2: Rehydration – The slides were kept in decreasing grades of alcohol (100%, 70% & 50%) for 5 minutes each followed by keeping them in running tap water for 5minutes

Step 3: Antigen retrieval – (pressure cooker method) 200 ml of clean tap water was taken in the empty pressure cooker & heated upto the steam formation. The slides were placed in the rack. 300 ml of ARB was put in the container & the rack with slides was placed inside the container. This container was then placed inside the pressure cooker & the lid was closed. After two whistles the pressure was released by lifting the air vent & allowed to cool till it reached the room temperature

Step 4: Washing – Slides were washed in Wash Buffer (pH7.4) thrice at a 1-minute interval

Step 5: Peroxide blocking–Blocking reagent was added to the sections & incubated for 10 minutes in a humidity chamber at room temperature. This step prevents unwanted, non-specific back ground staining

Step 6: The peroxide was decanted & not washed with buffer

Step 7: Primary antibodies – FGFR3 & VEGF were added separately to the sections & incubated in a humidity chamber for one hour

Step 8: Washing – Slides were washed in wash Buffer (pH 7.4) thrice at a 1-minute interval

Step 9: Post primary – Post primary was added over the sections & incubated for 10 minutes in a humidity chamber at room temperature

Step 10: Washing – The slides were washed in Wash Buffer (pH 7.4) thrice at a 1-minute interval

Step 11: HRP labelling – The HRP was added & incubated for 30 minutes in a humidity chamber at room temperature

Step 12: Washing – The slides were washed in Wash Buffer (pH 7.4)

thrice at a 1-minute interval

Step 13: DAB – The DAB chromogen was applied to the sections & incubated in the humidity chamber for 10 minutes, avoiding light exposure as much as possible

Step 14: Washing–The sections were washed in distilled water twice at a 1-minute interval

Step 15: Counter staining–Slides were counter stained using Harris Haematoxylin for 2-3minutes

Step 16: Washing–The slides were washed in running tap water for 5 minutes

Step 17: Dehydration–Slides were dehydrated by immersing in graded alcohol (50%, 70%, 95%, 100%), 1 minute each

Step 18: Mounting–Slides were air-dried, mounted with DPX & examined under the microscope

Membranous and cytoplasmic staining was seen in both FGFR3 and VEGF

Interpretation of IHC: A semi-quantitative (Q-score) scoring system was adopted for interpretation of IHC for FGFR3& VEGF

Q score = Intensity x Percentage staining.

- FGFR3

Percentage Score		**Intensity score**	
0	Negative or <5% tumor cells showing positivity	0	Negative
1+	5-25% tumor cells showing positivity	1+	Faint/detectable in some or all tumor cells
2+	26-50% tumor cells showing positivity	2+	Weak but extensive staining intensity
3+	51-75% tumor cells showing positivity	3+	Strong intensity
4+	>75% tumor cells showing positivity		

- Percentage & intensity were multiplied & grading was done as
  Score 0 & 1: Negative
  Score 2-12: Positive

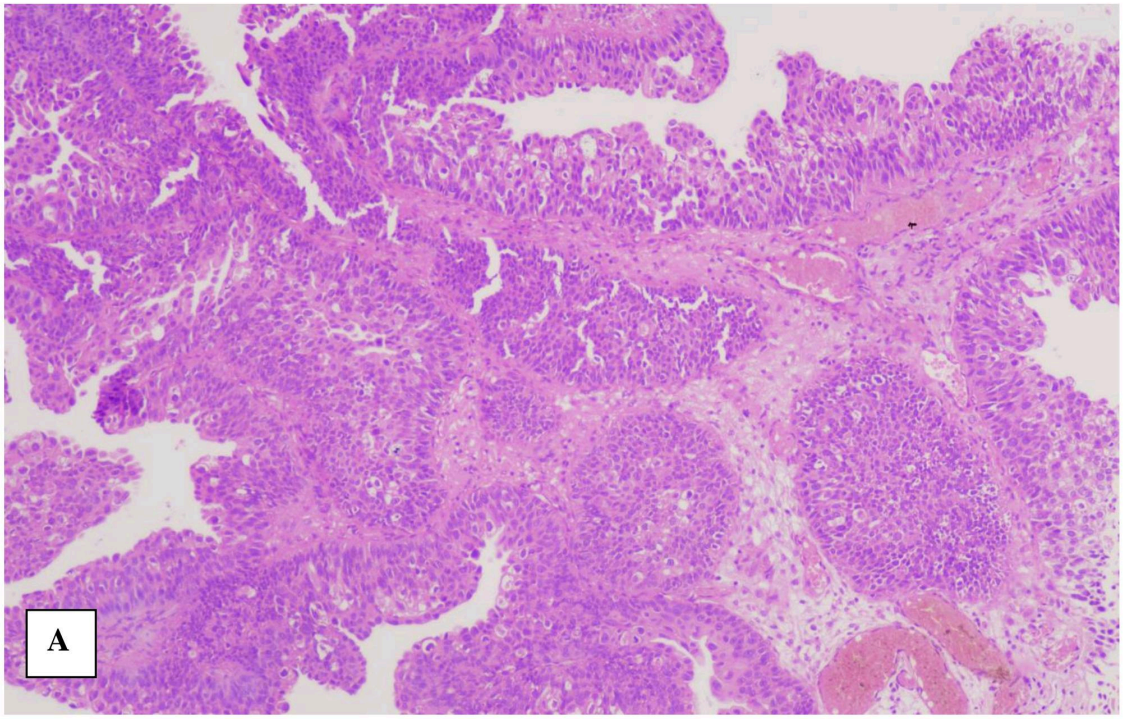
- VEGF

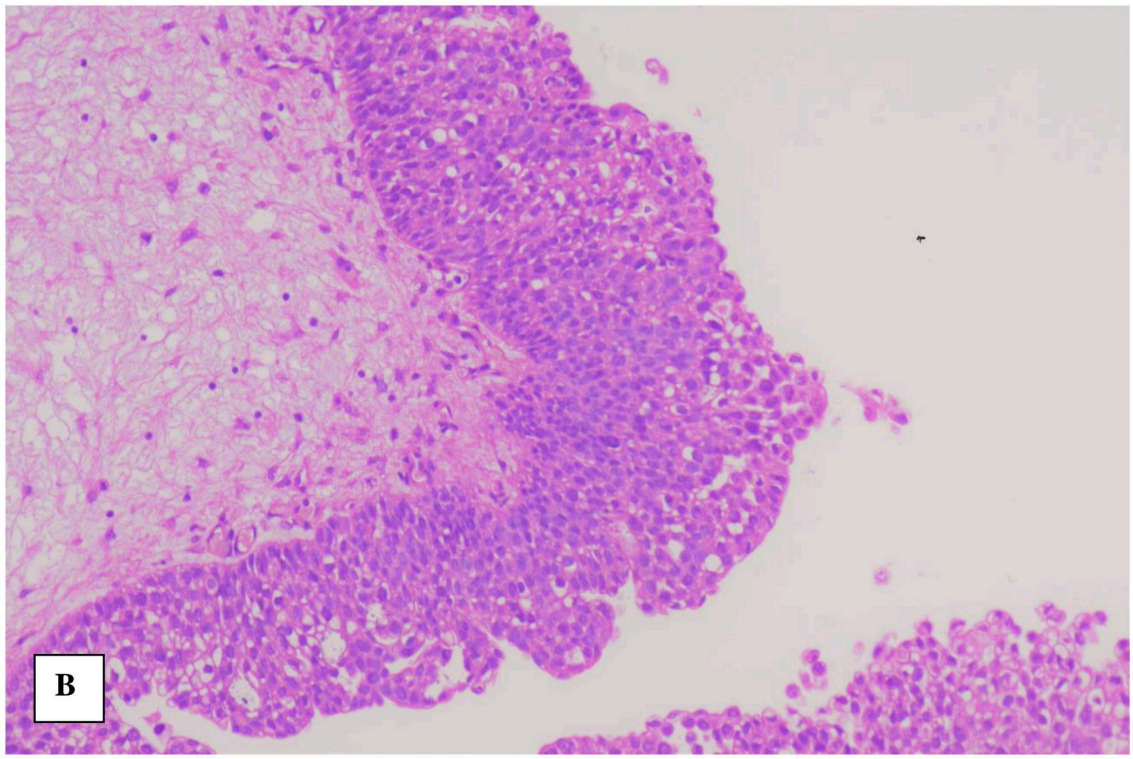
Percentage Score		Intensity score	
0	Negative or <5% tumor cells showing positivity	0	Negative
1+	5-25% tumor cells showing positivity	1+	Weak/faint in occasional tumor cells
2+	26-50% tumor cells showing positivity	2+	Weak but extensive staining in tumor cells
3+	51-75% tumor cells showing positivity	3+	Strong intensity
4+	>75% tumor cells showing positivity		

- Percentage & intensity were multiplied & grading was done as

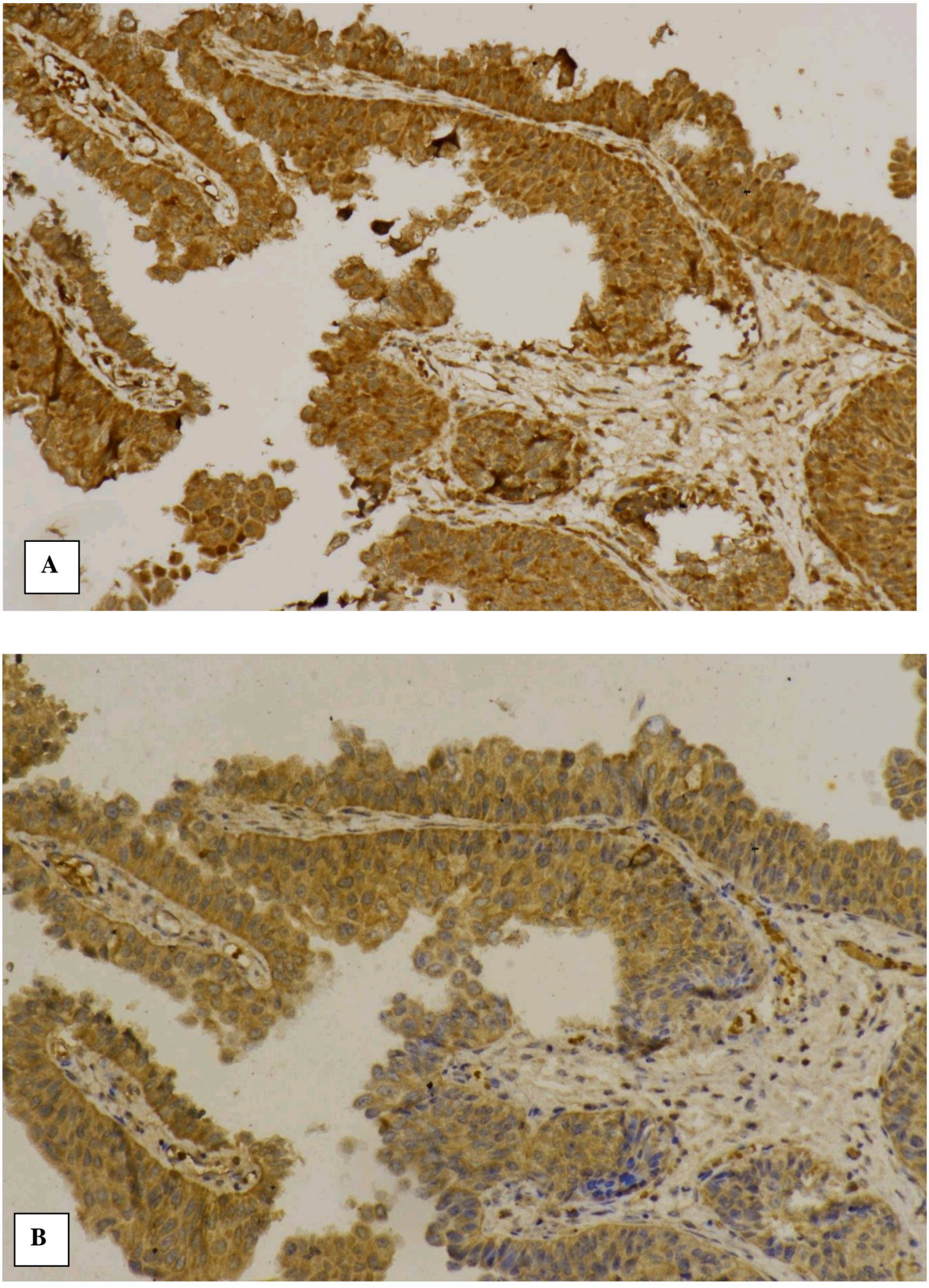
  Score 0 & 1: Negative

  Score 2-12: Positive

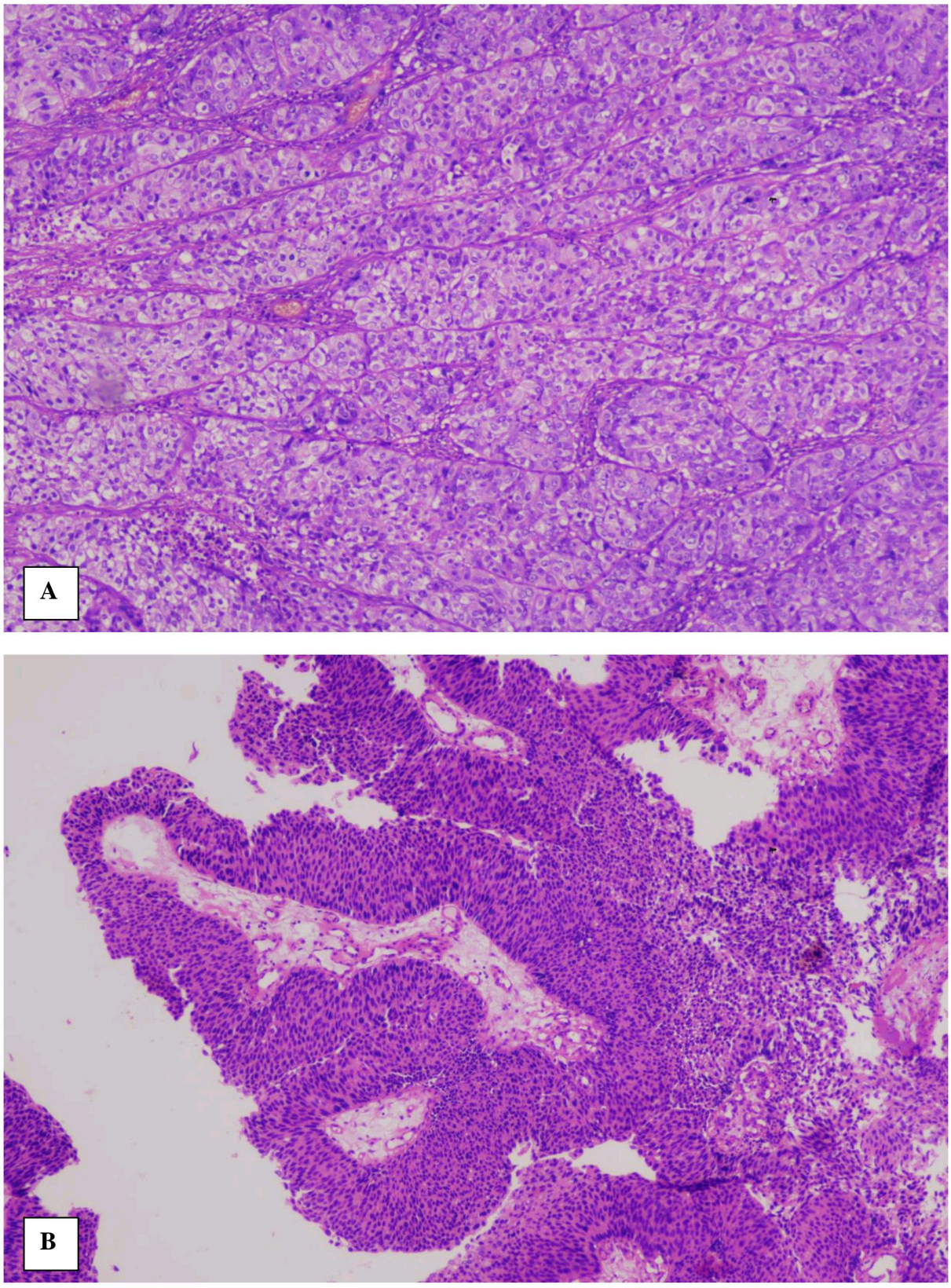




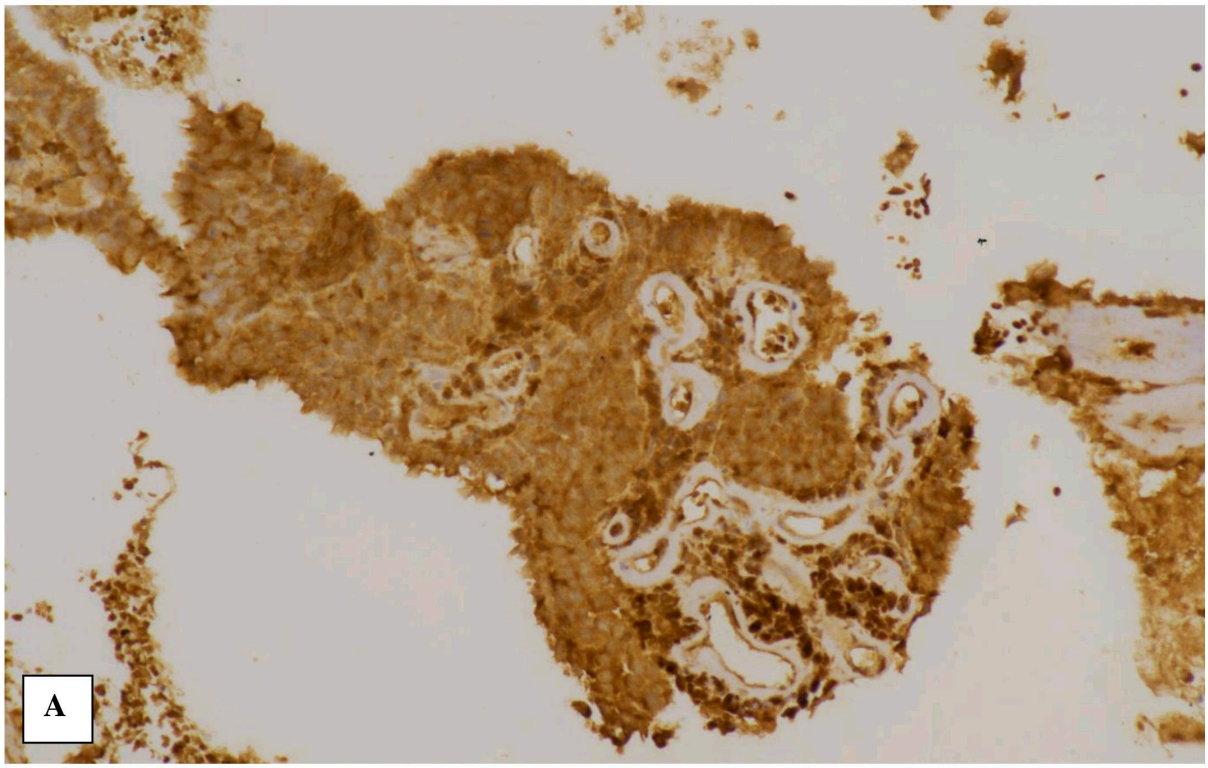
Photomicrograph 1: A & B shows a Low-grade non-invasive urothelial carcinoma (H&E 10x & 20x respectively).

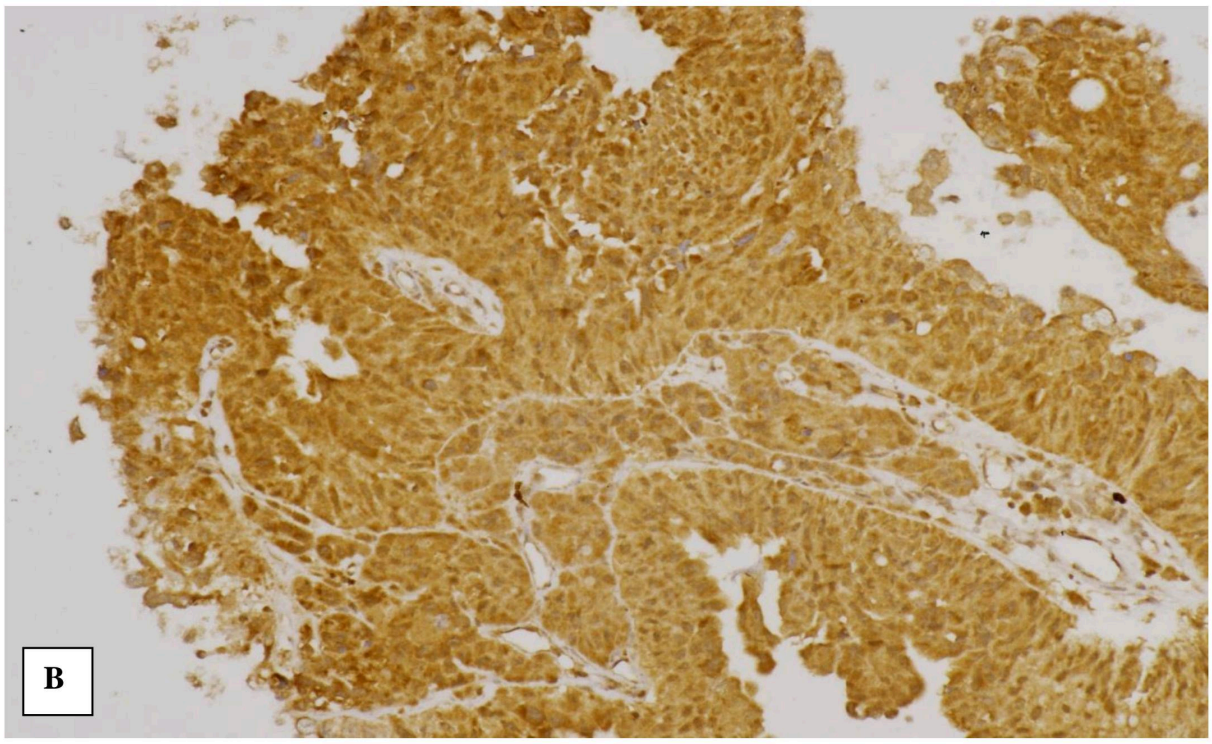


Photomicrograph 2: A & B display strong, intense membranous & cytoplasmic staining of the tumor cells (A: VEGF IHC 20x, B: FGFR3 IHC 20x)

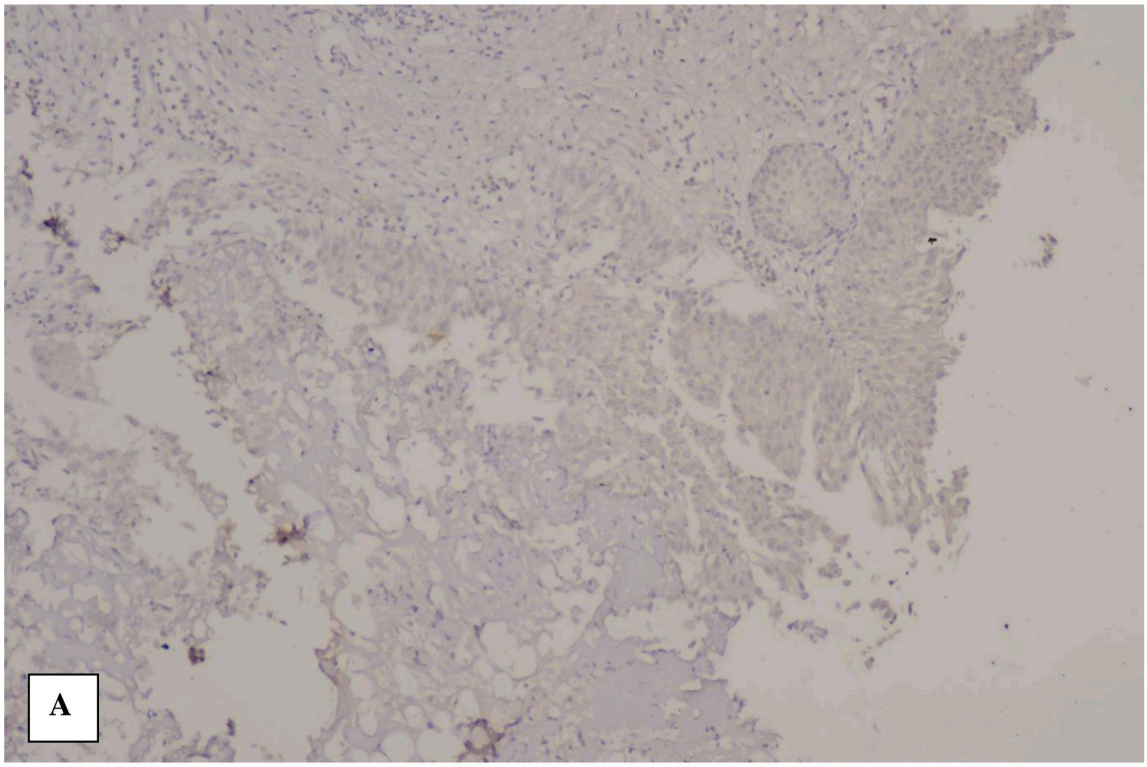


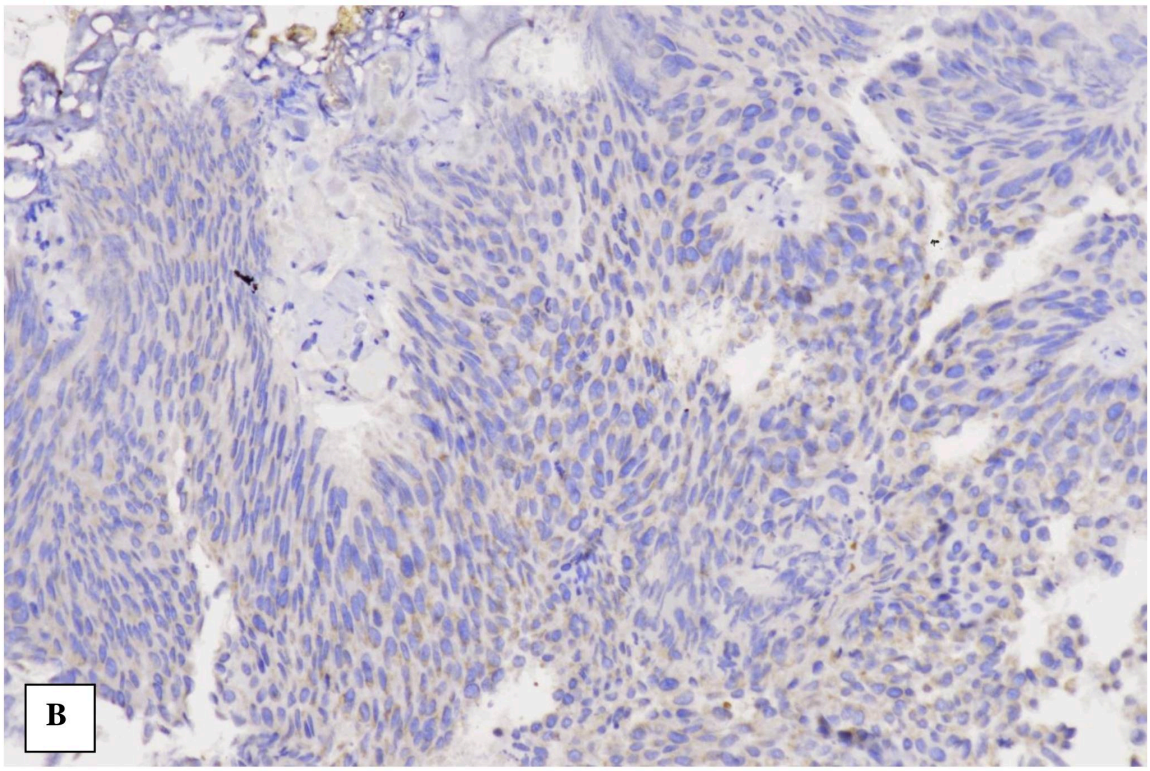
Photomicrograph 3: A. shows a high-grade, muscle-invasive urothelial carcinoma (H&E 40X). B. shows high-grade, non-muscle invasive urothelial carcinoma (H&E 10x)





Photomicrograph 4: A & B display strong, intense membranous & cytoplasmic staining of the tumor cells in High grade, non-muscle invasive urothelial carcinoma (A: VEGF IHC 20x, B: FGFR3 IHC 20x)





Photomicrograph 5: A and B does not show any staining (0) with the antibodies (A: FGFR3 IHC 10x, B: VEGF IHC 20x)

OBSERVATIONS & RESULTS

This was an ambispective hospital-based observational study conducted in All India Institute of Medical Sciences, Jodhpur during the period of three years i.e. July 2016 to July 2022. A total of 79 cases diagnosed as urothelial carcinomas, including muscle invasive & non-muscle invasive, both low grade & high grade were included in study.

AGE DISTRIBUTION OF CASES WITH MEAN & STANDARD DEVIATION

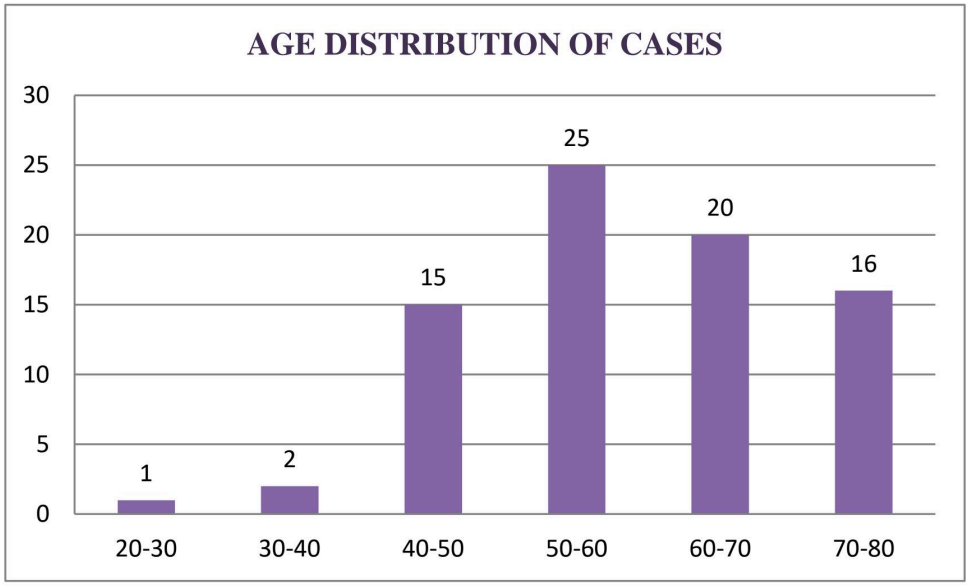


FIGURE 2

Urothelial carcinoma was observed in the elderly, with highest prevalence between the age group 50-60 years followed by 60-70 years. The mean age of occurrence was 61 years with a standard deviation of 13.41 years

GENDER WISE DISTRIBUTION OF CASES

SEX	NUMBER OF CASES (79)	PERCENTAGE
MALE	63	79.7%
FEMALE	16	20.3%

Table 1: Gender-wise distribution of cases

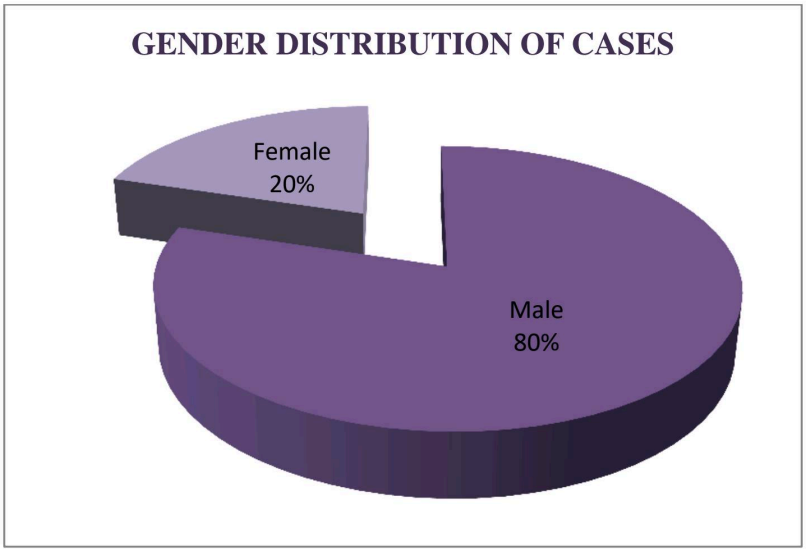


FIGURE 3

Of 79 cases, 63 were males & 16 females with a male to female ratio of 4:1

DISTRIBUTION OF SPECIMENS

TURBT	RADICAL CYSTOPROSTETECTOMY	NEPHROURETERECTOMY
72 (91.1%)	6 (7.6%)	1 (1.3%)

Table 2: Distribution of specimens (n=79)

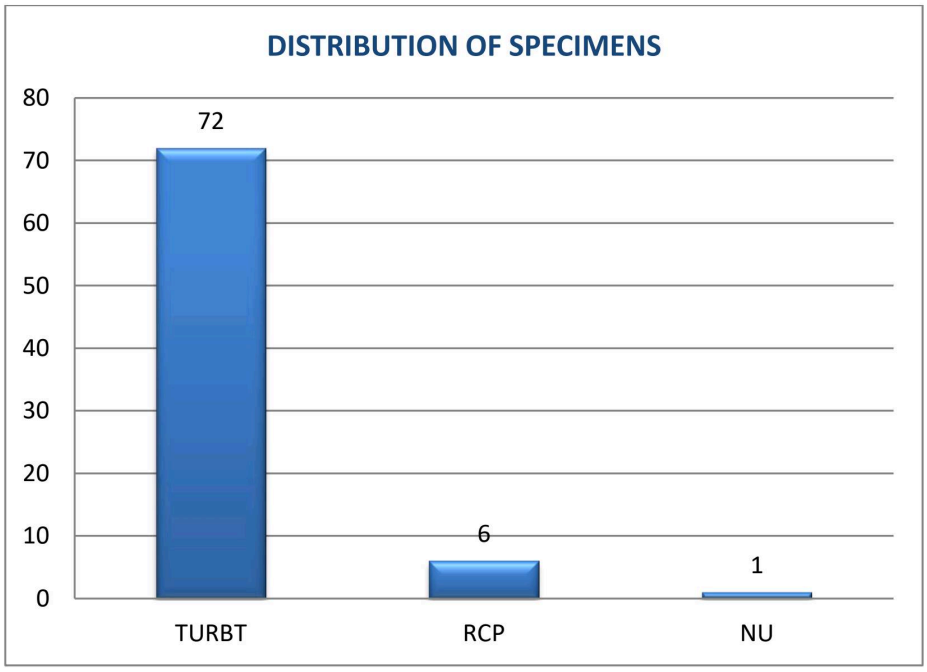


FIGURE 4: The distribution of specimens.

TURBT constitutes 91.1% of all the specimens received followed by radical cystoprostetectomies (7.5%) & nephroureterectomy constituting 1.2% of the specimens

DISTRIBUTION OF CASES ACCORDING TO TUMOR GRADE & TYPE

GRADE	NUMBER OF CASES (n=79)	PERCENTAGE
HIGH-GRADE	48	60.8%
LOW-GRADE	27	34.2%
SMALL CELL NEUROENDOCRINE CARCINOMA	1	1.3%
SQUAMOUS CELL CARCINOMA	3	3.8%

Table 3: Distribution of cases according to tumor grade & type

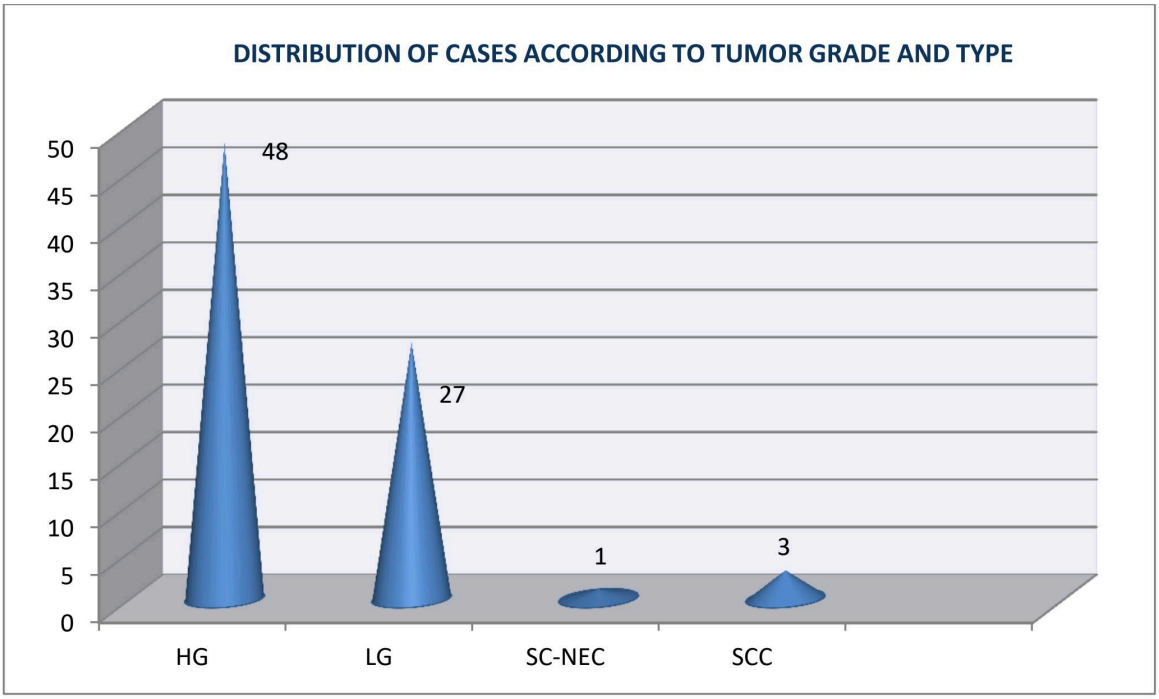


FIGURE 5

Out of the total 79 cases, 60.8% of cases were high-grade & 34.2% were low-grade urothelial carcinomas. Others constituted 5.1%, including small cell neuroendocrine carcinoma & squamous cell carcinoma.

DISTRIBUTION OF CASES ACCORDING TO SUBTYPES OF UROTHELIAL CARCINOMA

Papillary	Sarcomatoid	Nested	Plasmacytoid	Squamoid differentiation
68 (90.6%)	1 (1.33%)	1 (1.33%)	1 (1.33%)	4 (5.3%)

Table 4: Distribution of cases according to subtypes of urothelial carcinoma (n=75)

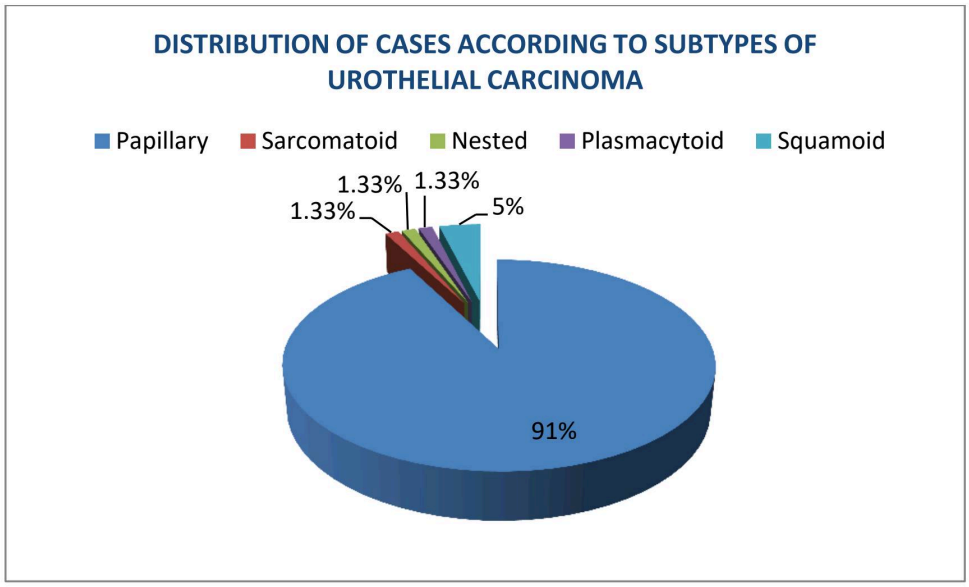


FIGURE 6

Out of 75 invasive & non-invasive urothelial carcinomas (n=75), 92.0% of cases were conventional papillary type, 4% cases showed squamoid differentiation; & sarcomatoid, nested & plasmacytoid subtypes accounted for 1.33% each respectively.

DISTRIBUTION OF CASES ACCORDING TO THE INVASION & STAGE

	NUMBER OF CASES (n=79)	PERCENTAGE (%)
INVASIVE (T1)	32	40.5
DEEP MUSCLE INVASION (T2)	12	15.2
NON-INVASIVE (Ta)	35	44.3

TABLE 5 - Distribution of cases according to the invasion

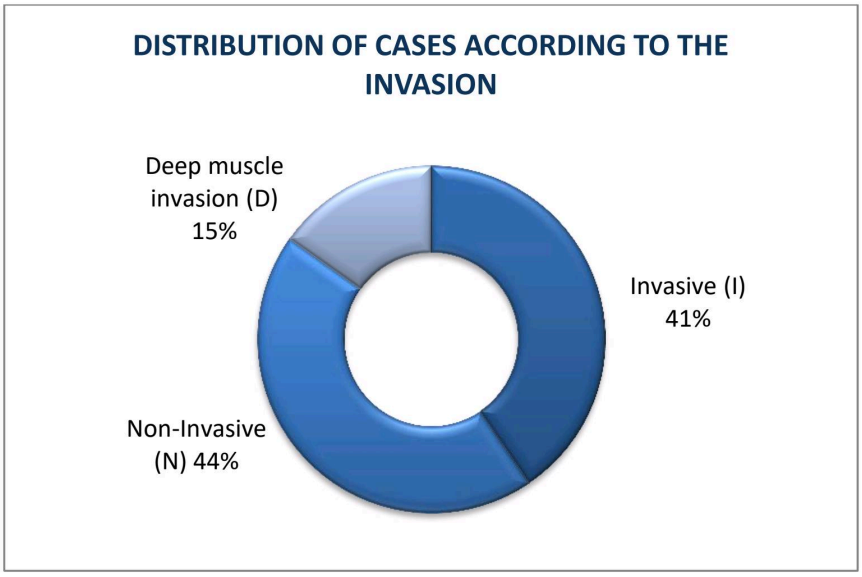


FIGURE 7

Among all the urothelial carcinomas (n=79), 35 (44.3%) cases were non-invasive, Ta; 32 (40.5%) cases showed lamina propria invasion, T1; 12 (15.2%) cases were infiltrating the deep muscle, T2

PERINEURAL INVASION IN UROTHELIAL CARCINOMA

PERINEURAL INVASION STATUS (PNI)	NUMBER OF CASES (n=79)	PERCENTAGE (%)
PNI	1	1.2
NO PNI	78	98.8

Table 6: Distribution based on perineural invasion status

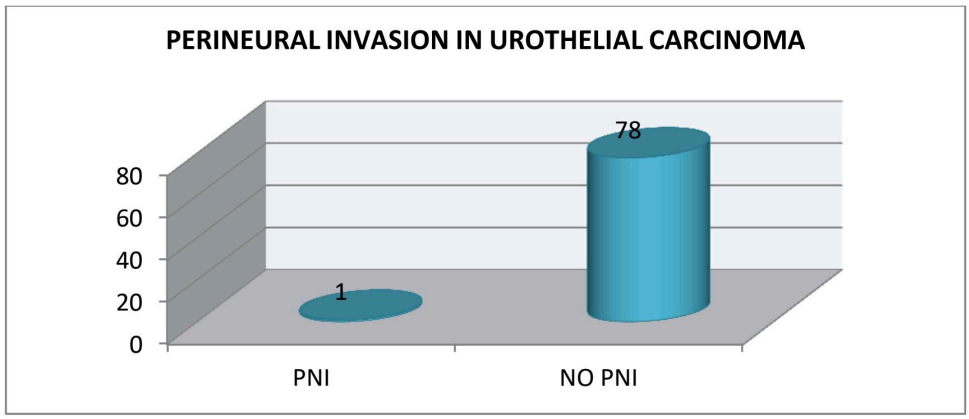


Figure 8: Distribution based on perineural invasion status.

Out of 79 cases only one case showed perineural invasion

LYMPHOVASCULAR INVASION IN UROTHELIAL CARCINOMA

LYMPHOVASCULAR INVASION	NUMBER OF CASES (n=79)	PERCENTAGE (%)
PRESENT	5	6.33
ABSENT	74	93.67

Table 7- Distribution bases on lymphovascular invasion status

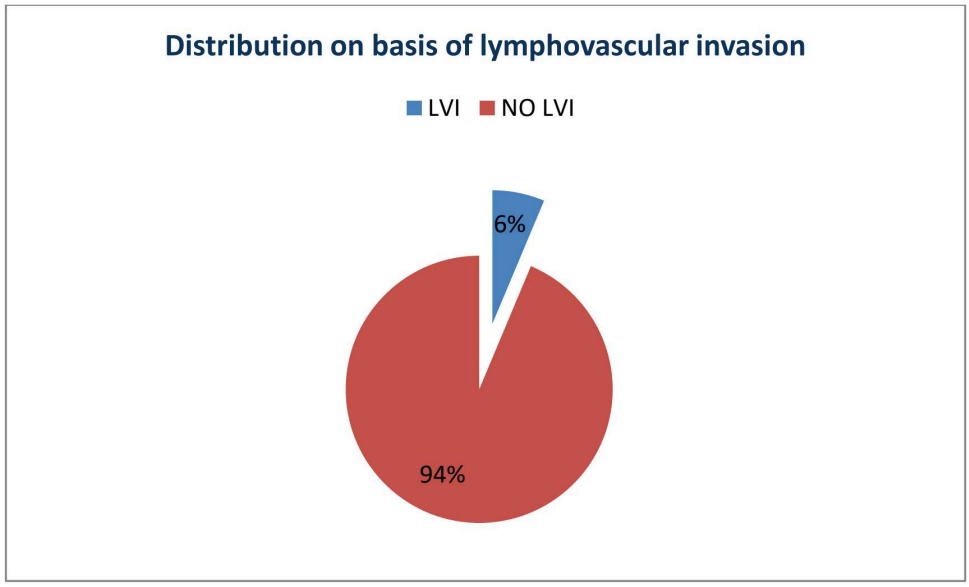


Figure 9

Out of 79 cases, 5 showed lymphovascular invasion. LVI was seen in 4 radical cystectoprostetectomy specimens & one TURBT specimen

FGFR3 EXPRESSION IN UROTHELIAL CARCINOMA

	NUMBER OF CASES (n=79)	PERCENTAGE (%)
POSITIVE	52	65.8
NEGATIVE	27	34.2

Table 8- Distribution of cases according to the expression of FGFR3

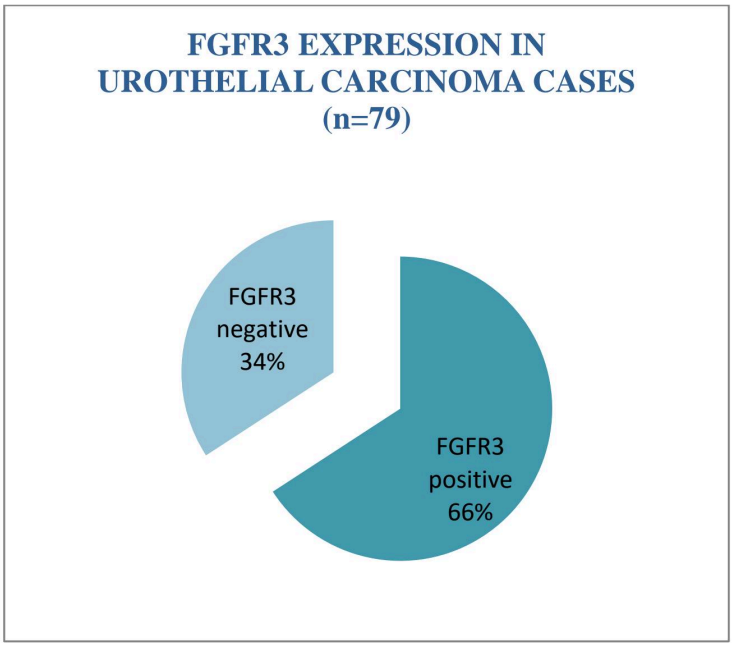


Figure 10

Of the n=79 cases, 52 showed positivity for FGFR3. Among these 52 cases, 28 cases showed strong cytoplasmic & membranous staining in >75% of the tumor cells & were given a score of 12. Rest 24 cases showed weak/ faint & weak but intensive staining in <75% of tumor cells & were given a score between 2-9.

Among the 27 negative cases, 17 cases showed no staining in any of the tumor cells. However, 10 cases showed weak / faint staining in <25 % tumor cells & were given a score of 1.

VEGF EXPRESSION IN UROTHELIAL CARCINOMA

	NUMBER OF CASES (n=79)	PERCENTAGE (%)
POSITIVE	64	81.0
NEGATIVE	15	19.0

Table 9- Distribution of cases according to the expression of VEGF

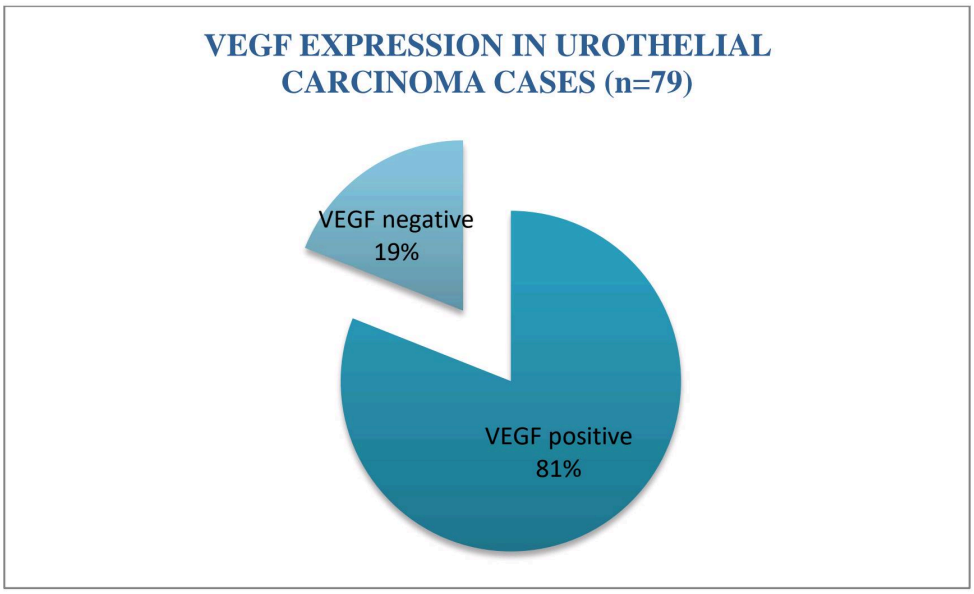


FIGURE 11

Of the n=79 cases, 64 exhibited cytoplasmic & membranous positivity for VEGF. Among these 64 positive cases, 40 showed strong cytoplasmic & membranous staining in >75% of the tumor cells & were given a score of 12. Rest 24 cases showed weak/ faint & weak but intensive staining in <75% of tumor cells & were given a score between 2-9.

Of the 15 negative cases, 12 did not show any staining in the tumor cells. However, 3 cases showed weak/faint staining in <25% tumor cells & were given a score of 1.

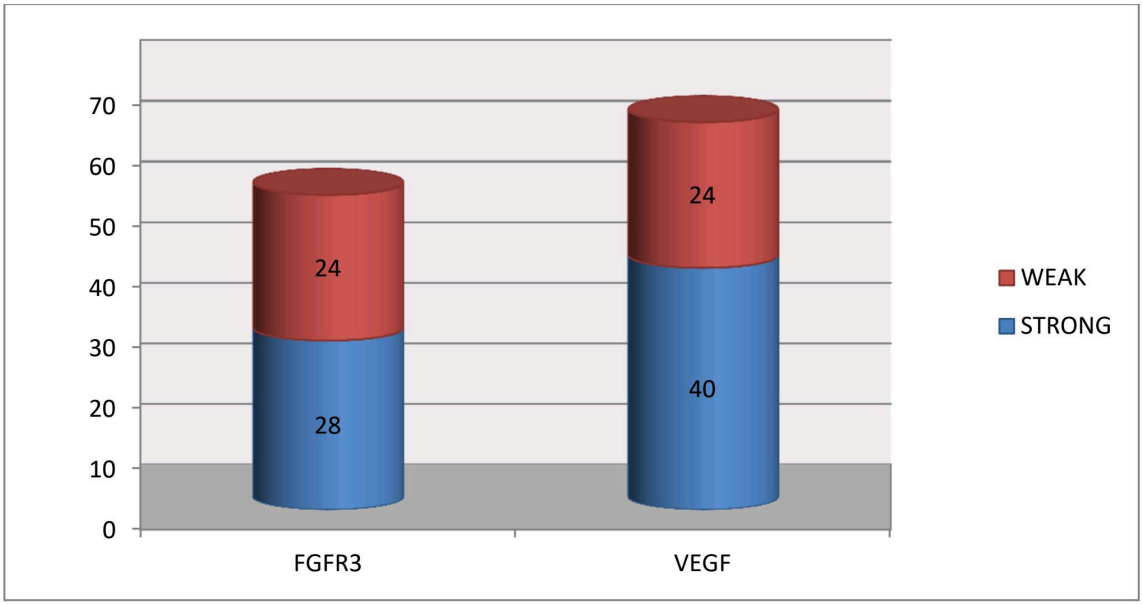


FIGURE 12

Distribution of cases according to the intensity of cytoplasmic & membranous staining.

CORRELATION OF FGFR3 & VEGF EXPRESSION WITH VARIOUS PARAMETERS

		DIAGNOSIS							
		HGUC (n=48)		LGUC (n=27)		SC-NEC (n=1)		SCC (n=3)	
		CASES	%	CASES	%	CASES	%	CASES	%
FGFR3 INTERPRETATION RESULT	N	16	59.3	8	29.6	1	3.7	2	7.4
	P	32	61.5	19	36.6	0	0.0	1	1.9
VEGF INTERPRETATION RESULT	N	9	60.0	5	33.4	0	0.0	1	6.7
	P	39	60.9	22	34.4	1	1.6	2	3.1

TABLE 10: Correlation of FGFR3 & VEGF with respect to the grade & type of urothelial carcinoma.

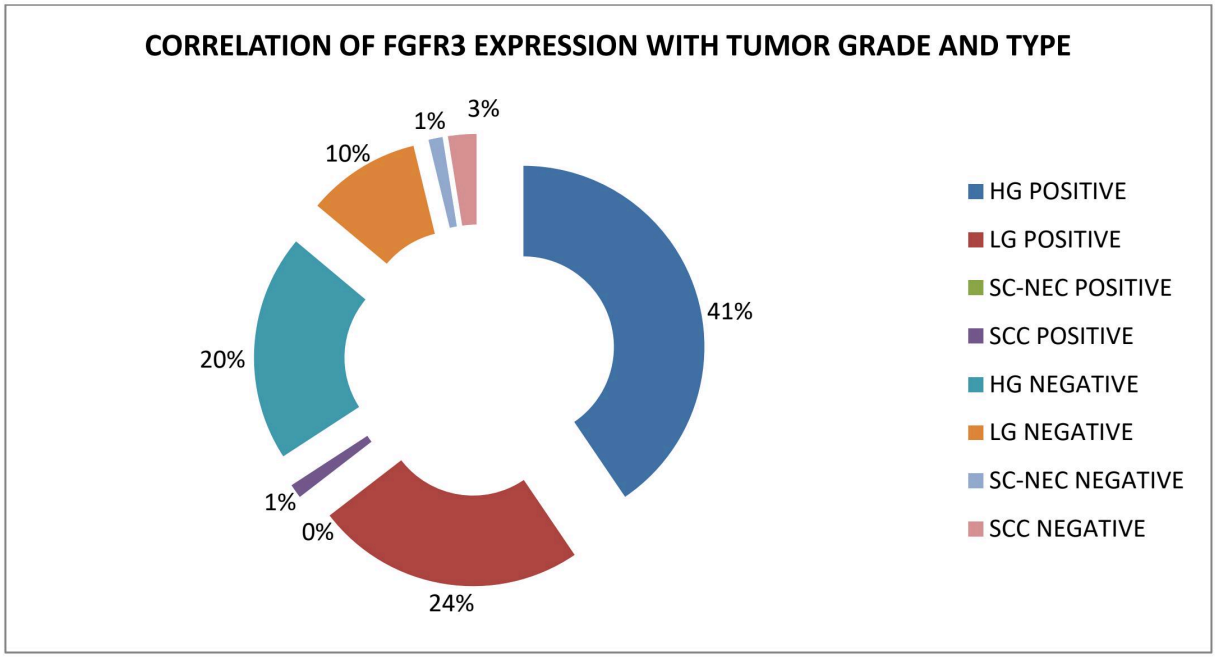


FIGURE 13

FGFR3 expression in all the 79 cases of urothelial carcinomas.

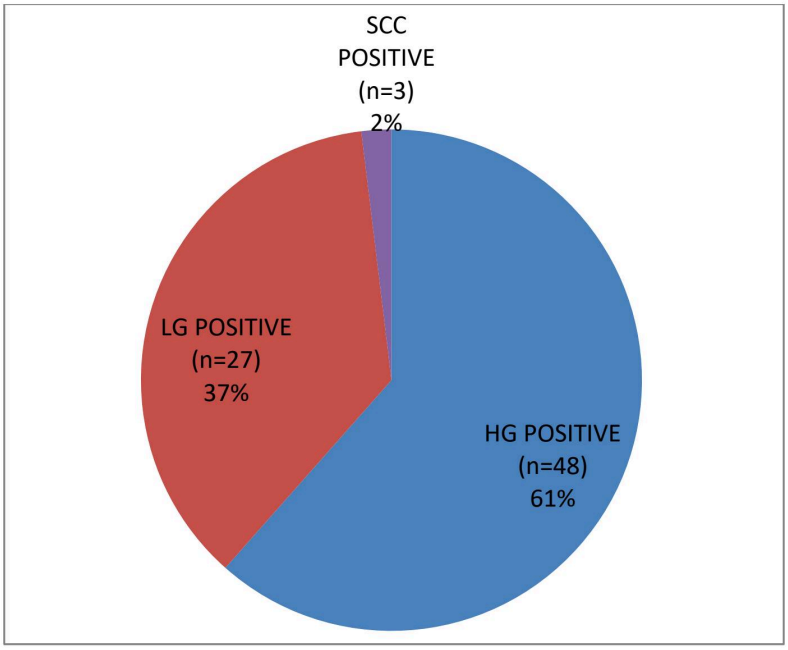


FIGURE 14: Distribution of FGFR3 positive cases.

FGFR3 was positive in 52 cases (65.8%) out of which 61.5% were HGUC, 36.6% were LGUC & 1.9% was SCC.

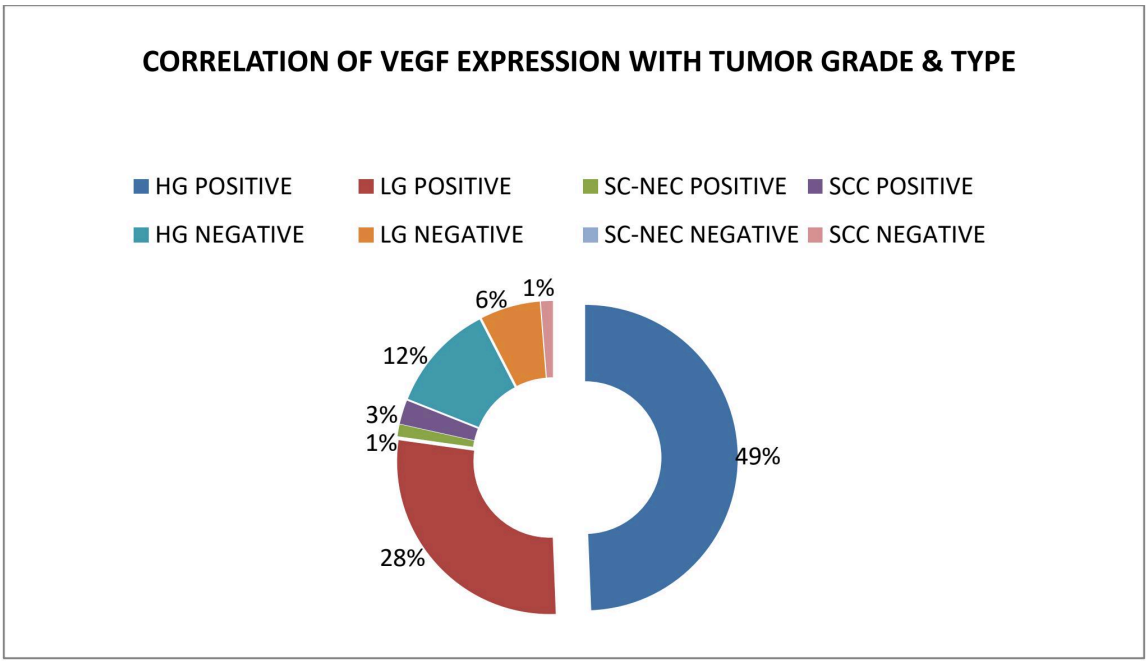


FIGURE 15

VEGF expression in all the 79 cases of urothelial carcinomas. Out of these, 64 (81%) cases showed expression of VEGF.

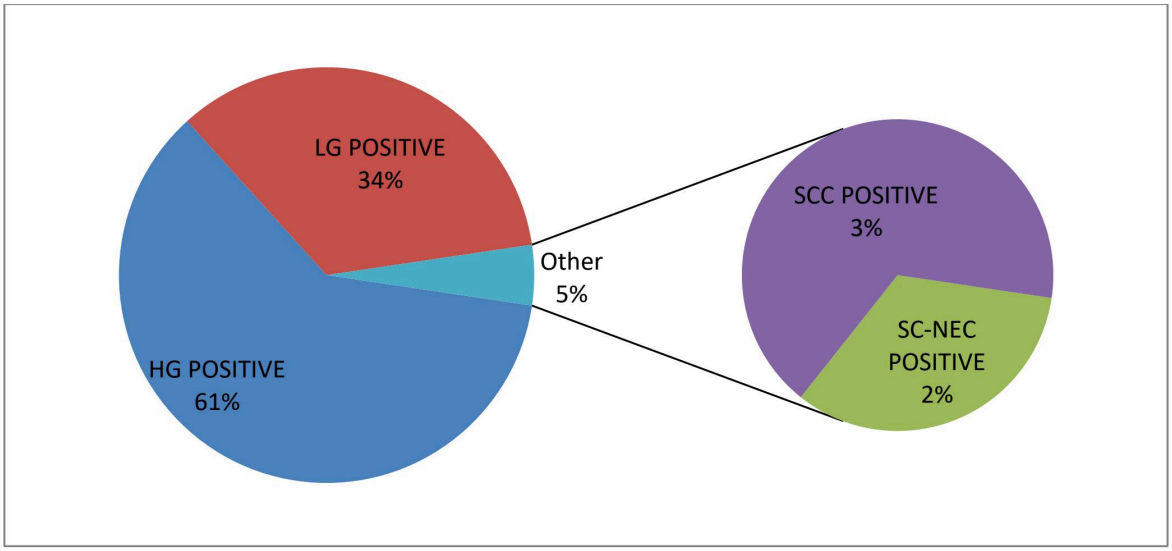


FIGURE 16: Distribution of VEGF positive cases.

VEGF was positive in 60.9% HGUC, 34.4% LGUC, 3.1% SCC & 1.6% SC-NEC

FGFR3 INTERPRETATION RESULT			INVASION					
			D (n=12)		I (n=32)		N (n=35)	
			CASES	%	CASES	%	CASES	%
NEGATIVE	DIAGNOSIS	HG	5	31.3	6	37.5	5	31.3
		LG	0	0.0	3	37.5	5	62.5
		SC-NEC	0	0.0	1	100.0	0	0.0
		SCC	0	0.0	2	100.0	0	0.0
POSITIVE	DIAGNOSIS	HG	7	21.9	17	53.2	8	25.0
		LG	0	0.0	3	15.8	16	84.2
		SCC	0	0.0	0	0.0	1	100.0

TABLE 11

Correlation of FGFR3 with respect to grade & stage of urothelial carcinoma

(D-deep muscle invasive, I-lamina propria invasion, N- Non-invasive)

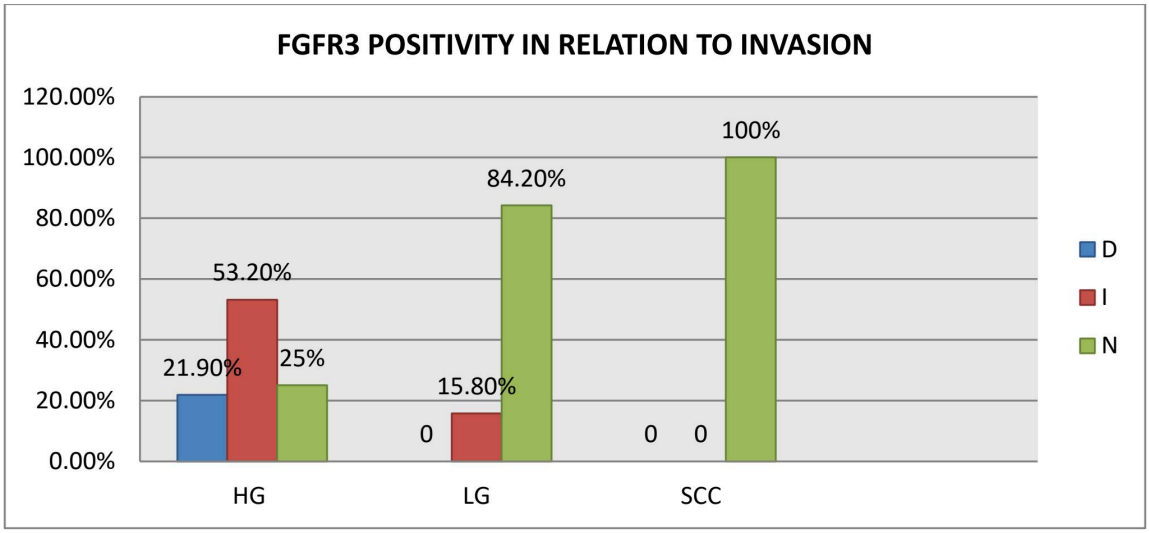


FIGURE 17: FGFR3 positive urothelial carcinoma with respect to the stage (invasion).

Among HGUC, 8 (25%) were non-invasive,Ta; 17 (53.2%) were showing lamina propria invasion, T1; 7 (21.9%) were deep muscle invasive (T2). Within LGUC, 16 (84.2%) were non-invasive, Ta & only 3 (15.8%) showed lamina propria invasion.

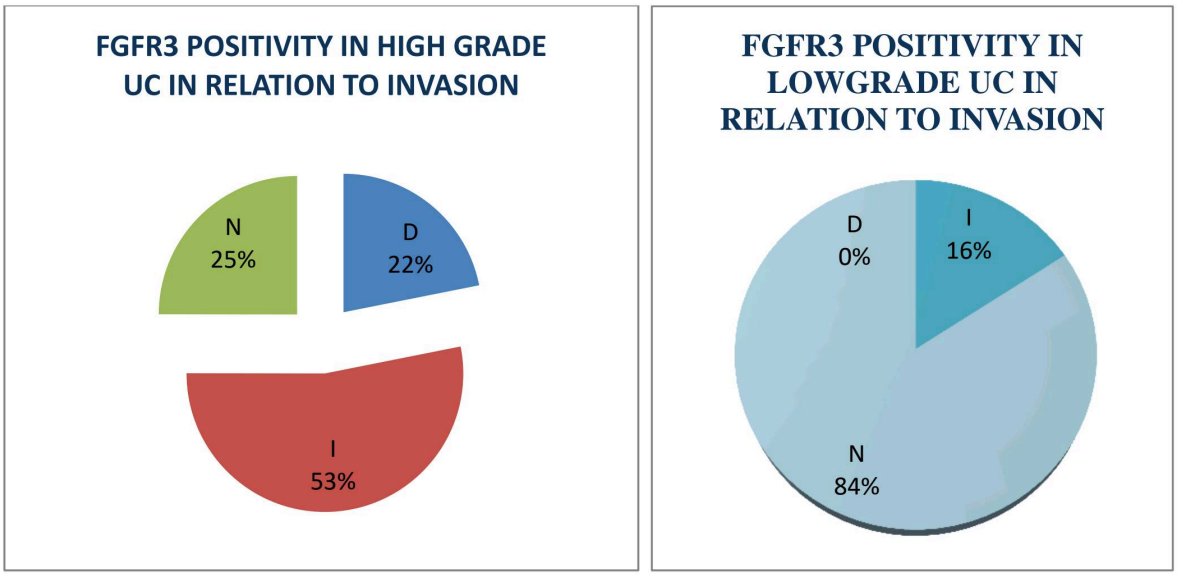


FIGURE 18

Distribution of FGFR3 positive HGUC & LGUC with respect to stage (invasion: D-deep muscle invasive, I-lamina propria invasion, N- Non-invasive)

VEGF INTERPRETATION RESULT			INVASION					
			D (pT2)		I (pT1)		N (pTa)	
			CASES	%	CASES	%	CASES	%
NEGATIVE	DIAGNOSIS	HG	3	33.3	3	33.3	3	33.3
		LG	0	0.0	0	0.0	5	100.0
		SCC	0	0.0	0	0.0	1	100.0
POSITIVE	DIAGNOSIS	HG	9	23.1	20	51.3	10	25.6
		LG	0	0.0	6	27.3	16	72.7
		SC-NEC	0	0.0	1	100.0	0	0.0
		SCC	0	0.0	2	100.0	0	0.0

TABLE 12: Correlation of VEGF with respect to grade & stage of urothelial carcinoma (D-deep muscle invasive, I-lamina propria invasion, N- Non-invasive)

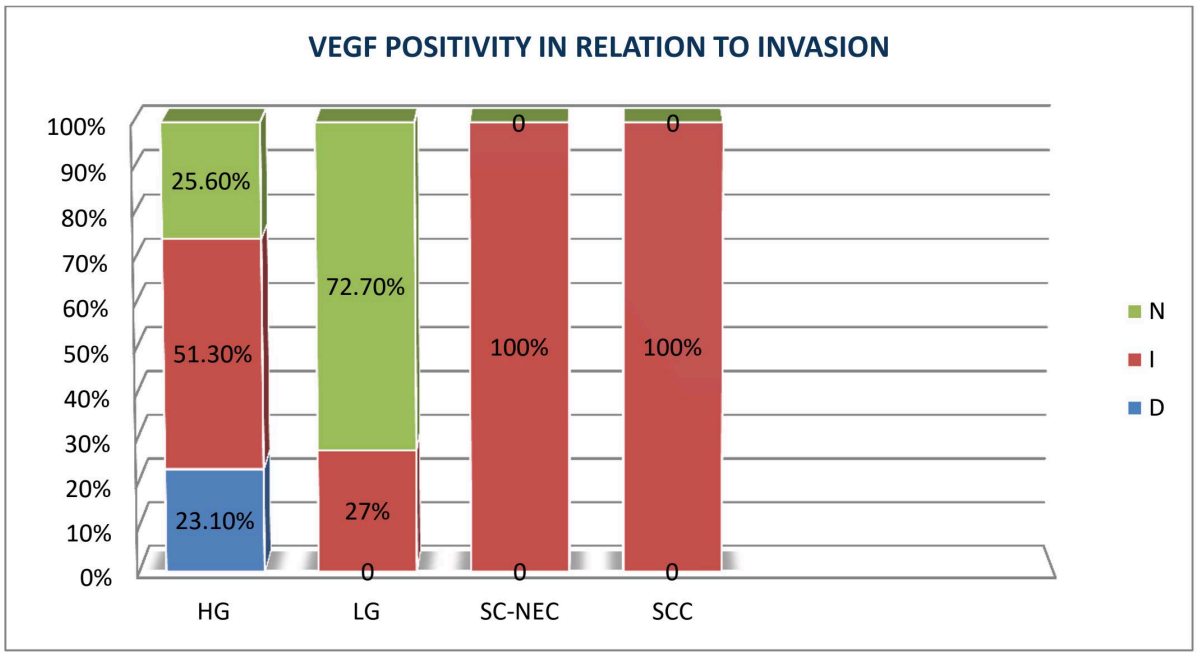


FIGURE 19:

VEGF positive urothelial carcinoma with respect to the stage (invasion). Among HGUC, 10 (25.6%) were non-invasive,Ta; 20 (51.3%) showed lamina propria invasion, T1& 9 (23.1.%) were deep muscle invasive (T2). Within LGUC, 16 (72.7%) were non-invasive, Ta & only 6 (27%) showed lamina propria invasion. 2 positive cases of SCC & one case of SC-NEC showed lamina propria invasion.

SUMMARY OF THE RESULTS

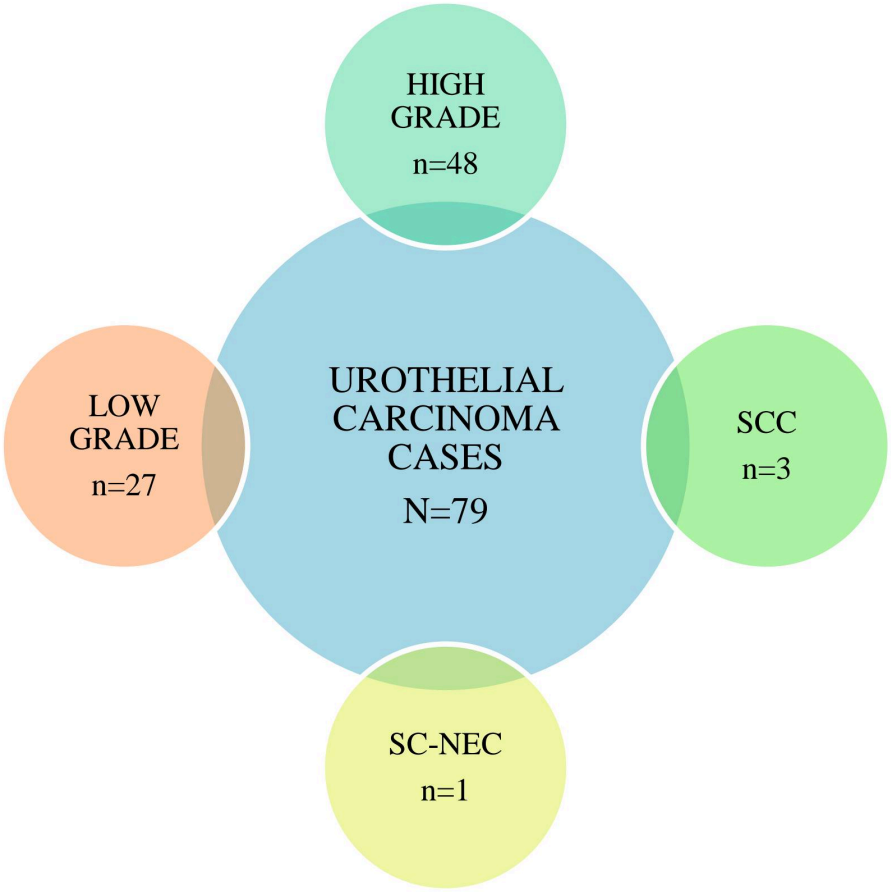


FIGURE 20

The present study included n=79 cases, of which 75 were conventional papillary urothelial carcinomas (HG & LG), 3 were squamous cell carcinomas & 1 was neuroendocrine carcinoma.

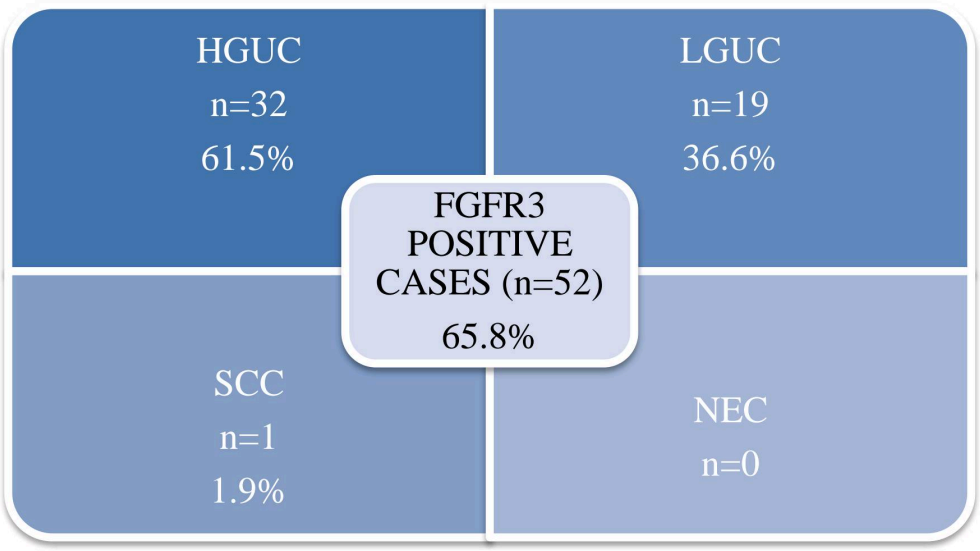


FIGURE 21

Of the 79 cases, 52 showed positive FGFR3 expression, which included 32 (61.5%) HGUC cases, 19 (36.6%) LGUC cases, & 1 (1.9%) case of SCC.

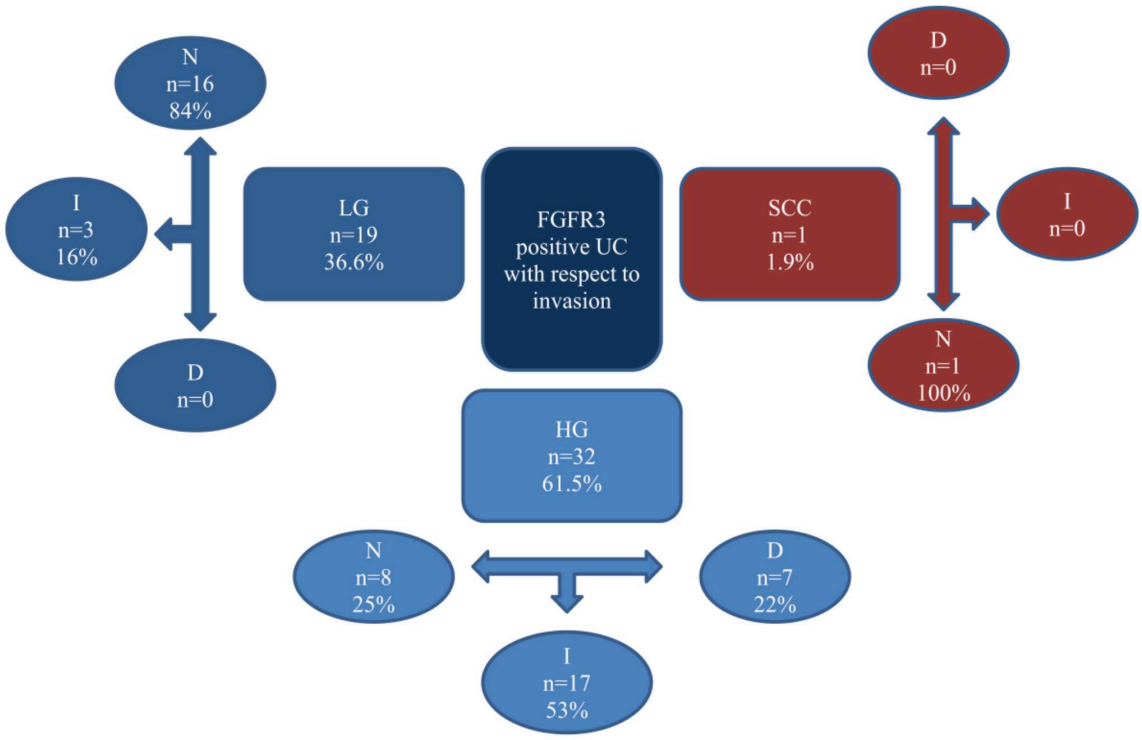


FIGURE 22

Distribution of FGFR3 positive cases based on the Grade & stage of urothelial carcinoma

FGFR3 INTERPRETATION RESULT: MUSCLE INVASIVE & NON-MUSCLE INVASIVE UROTHELIAL CARCINOMA					
			Invasive Non-Invasive		Total
			D	I	
FGFR3 INTERPRETATION RESULT	N	Count	5	22	27
		% within FGFR3 INTERPRETATION RESULT	18.5%	81.5%	100.0%
	P	Count	7	45	52
		% within FGFR3 INTERPRETATION RESULT	13.5%	86.5%	100.0%
Total		Count	12	67	79
		% within FGFR3 INTERPRETATION RESULT	15.2%	84.8%	100.0%

TABLE 13:

FGFR3 INTERPRETATION RESULT: MUSCLE INVASIVE & NON-MUSCLE INVASIVE UROTHELIAL CARCINOMA

Out of these (n=79), 52 cases showed FGFR3 positivity & among these 32 (61.5%) cases were HGUC, which included 8 (25%) non-invasive HGUC, 17 (53%) lamina propria invasive HGUC & 7 (22%) deep muscle invasive HGUC. 19 (36.6%) cases of LGUC showed FGFR3 positivity. Of these 16 (84%) were non-invasive LGUC & 3 (16%) showed lamina propria invasion. No deep muscle invasive FGFR3 positive LGUC was seen.

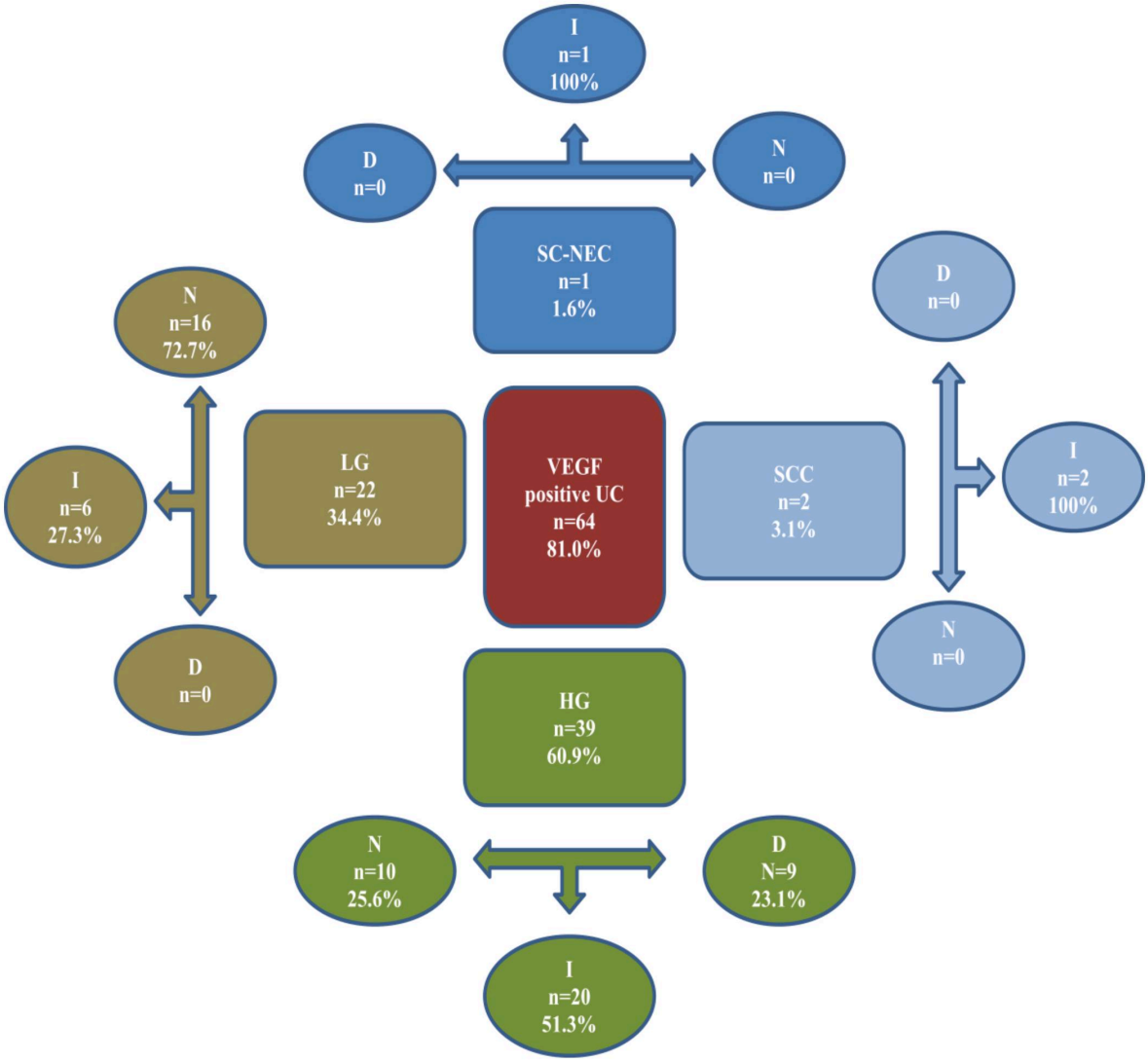


FIGURE 23

Distribution of VEGF positive cases based on the Grade, stage & types of urothelial carcinoma

VEGF INTERPRETATION RESULT: MUSCLE INVASIVE & NON-MUSCLE INVASIVE UROTHELIAL CARCINOMA					
			Invasive Non-Invasive		Total
			D	I	
VEGF INTERPRETATION RESULT	N	Count	3	12	15
		% within VEGF INTERPRETATION RESULT	20.0%	80.0%	100.0%
	P	Count	9	55	64
		% within VEGF INTERPRETATION RESULT	14.1%	85.9%	100.0%
Total		Count	12	67	79
		% within VEGF INTERPRETATION RESULT	15.2%	84.8%	100.0%

TABLE 14: VEGF INTERPRETATION RESULT: MUSCLE INVASIVE & NON-MUSCLE INVASIVE UROTHELIAL CARCINOMA

Out of total 79 cases, 64 cases showed VEGF positivity & among these 39 (60.9%) cases were HGUC, which included 10 (25.6%) non-invasive HGUC, 20 (51.3%) lamina propria invasive HGUC & 9 (23.1%) deep muscle invasive HGUC. VEGF positive 22 (34.4%) LGUC included 16 (72.7%) non-invasive LGUC & 6 (27.3%) lamina propria invasive LGUC. No deep muscle invasive VEGF positive LGUC was seen.

DISCUSSION

The main subject of study, in context to bladder tumors, is the research for new prognostic factors & molecular markers that are associated with the diagnosis & the prognosis of bladder cancers. As we know, the Fibroblast growth factor receptor 3 (FGFR3) is involved in the regulation of proliferation, differentiation, & apoptosis(38). It has been seen that the noninvasive urothelial carcinomas of the bladder harbor FGFR3 gene mutation & correlate with better clinical outcome(39).

One of the fundamental processes in tumor growth & metastasis is angiogenesis. VEGF is one of the potent stimulator of angiogenesis & a key mediator of angiogenesis in cancer. In many types of human tumors, vascular endothelial growth factor (VEGF) expression has been documented as prognostic indicator(30).

Age distribution of urothelial carcinoma cases

In our study n= 79 cases were included with the mean age of presentation being 61 years which was in concordance with the study by Gupta et al who included 561 patients & the mean age was 60.5(47). As per the American cancer society 2020 data, 73 years is the mean age at the time of diagnosis of urothelial carcinoma & approximately 95% cases are above the age of 55 years(48). The present study also had similar results with higher rates of urothelial carcinoma in the elderly.

Gender distribution

The GLOBOCAN (Global Cancer Incidence, Mortality and Prevalence) database 2020 states that bladder cancer is around four times more likely to be diagnosed in men than women(2). In the study by Malik et al, the male to female ratio was 4:1(6) The present study also showed similar data, showing higher prevalence of urothelial carcinoma in males (79.7%) as compared to females (20.3%).

Specimen distribution

In the present study, 91.1% of the cases were TURBT specimens, 7.6% were radical cystoprostetectomies & 1.3% were nephroureterectomy specimens. This is attributed to the treatment modalities of urothelial carcinomas wherein TURBT is done for diagnosis and evaluation of deep muscle invasion, followed by relevant treatment in the form of intravesical BCG therapy or surgical intervention.

Tumor grade & stage

Our study was an ambispective hospital based observational study conducted at All India Institute of Medical Sciences, Jodhpur during from July 2020 to July 2022 in patients diagnosed with urothelial carcinoma. 79 cases fulfilled the inclusion criteria & were included in study.

The aim of the present study was to determine the association of FGFR3 & VEGF in patients with urinary bladder carcinoma, both invasive & non-invasive, & to analyze their prognostic value.

In 2013, Chou et al. found that incidence of MIBC is higher; revealing 40.5% NMIBC and 59.5% MIBC(41). However, in 2016 the study conducted by Chinnasamy et al, recorded 86.5% cases of NMIBC (stage- PT1) & 13.5% cases of MIBC (stage- PT2)(42). Thapa et al, in 2017, observed muscle invasion in 24.45% cases of high-grade urothelial carcinomas & none of the low grade papillary urothelial carcinoma cases included in their study showed muscle invasion(43). In present study, twenty-seven cases were of low grade urothelial carcinoma (n=27) & all of these were non-muscle invasive bladder carcinomas. This can be attributed to the low sample size. On the other hand, among 48 cases of high-grade carcinomas (n=48), 75% were NMIBC & 25% were MIBC. Incidence of NMIBC was high in our study.

TABLE 15: SUMMARY OF DIFFERENT STUDIES

AUTHOR	STUDY	STUDY PERIOD	NUMBER OF CASES
Laura S.Mertens et al (24)	Prognostic markers in invasive bladder cancer: *FGFR3* mutation status versus P53 and KI-67 expression	1986-2016	1058 CASES
Anika Sadaf et al(23)	Significance of Vascular Endothelial Growth Factor Expression in the Bladder Urothelial Carcinoma and Its Association with Tumor Grade and Invasiveness	2018-2020	56 CASES
Bas W.G. van Rhijn et al(40)	FGFR3 Mutation Status and FGFR3 Expression in a Large Bladder Cancer Cohort Treated by Radical Cystectomy	1986-2016	1000 CASES

Malik et al(6)	Role of FGFR3 in Urothelial Carcinoma	2013-2015	55 CASES
Behl et al(28)	Expression of VEGF in patients of urinary bladder carcinoma	1 YEAR	50 CASES
Arshad Rahmani et al(30)	Expressional evaluation of Vascular Endothelial Growth Factor (VEGF) protein in urinary bladder carcinoma patients exposed to cigarette smoke	-	125 CASES
Young-Hee Maeng et al(31)	Expression of Fibroblast Growth Factor Receptor 3 in the Recurrence of Non-Muscle-Invasive Urothelial Carcinoma of the Bladder	2001-2007	55 CASES
J Javier Gómez-Román et al(27)	Fibroblast growth factor receptor 3 is overexpressed in urinary tract carcinomas and modulates the neoplastic cell growth	2005	237 CASES
Ching-ChiangYang(33)	The expression of vascular endothelial growth factor in transitional cell carcinoma of urinary bladder is correlated with cancer progression	2004	161 CASES
DC Tomlinson(32)	FGFR3 protein expression and its relationship to mutation status and prognostic variables in bladder cancer	2004-2005	158 CASES
Present study	Expression of Fibroblast Growth Factor Receptor (FGFR3) & Vascular Endothelial Growth Factor (VEGF) In Malignant Tumors of the Urothelial tract	2020-2022	79 CASES

Lymphovascular invasion and perineural invasion

In the present study lymphovascular invasion was seen in 5 (6.3%) cases (n=79), of which 4 cases were high grade urothelial carcinomas & 1 was small cell neuroendocrine carcinoma. All of these cases, irrespective of the invasion status, showed positive VEGF expression. However, only 2 cases showed positive FGFR3 expression, which included 1 muscle invasive & 1 non-muscle invasive urothelial carcinoma.

Out of 79 cases only one case showed PNI which was positive for VEGF.

FGFR3 expression in urothelial carcinoma

In the Indian population, Malik et al(6) showed FGFR3 overexpression by immunohistochemistry in 24/32 cases (78.1%) of non-invasive urothelial cancers & 4/22 (18.2%) cases of invasive urothelial carcinomas. In our study, FGFR3 was positive in 86.5% cases of non-muscle invasive urothelial carcinomas (pTa &pT1) & 13.5% cases of muscle invasive urothelial carcinoma. This is in concordance with the other studies done (31,32,43) & confirms that FGFR3 mutations are found predominantly present in low grade urothelial carcinomas. Mertens et al(24) determined the mutation in FGFR3 gene by using PCR-SNaPshot along with p53 & Ki-67 expression by IHC. According to their study FGFR3 mutation was detected in 107 (10%) of the cases & was associated with lower pT-stage.

TABLE 16: FGFR3 EXPRESSION IN VARIOUS STUDIES

AUTHOR	CASES	FGFR3 EXPRESSION (%)	ASSOCIATED STAGE & GRADE
Laura S Mertens et al (24)	1058	-10	-pTa-1 & LG
Rhijn et al(40)	1000	-28	-pTa-1 & LG
Alec Kacew & Randy F. Sweis et al(44)	-	-49-84 -18	-pTa-1 & LG -pT2
Malik et al(6)	55	-66.7 -82.6 -18	-pTa & HG -pTa & LG -pT1 & HG
Young-Hee Maeng et al(31)	55	-81.3 -47.8 -78.9 -41.2	-LG -HG -pTa -pT1
Tomlinson et al(32)	158	-73	-pTa
Present study	79	-86.5 -13.5	-pTa-1 -pT2

In our study, 39 cases (81.3%) of high-grade urothelial carcinomas (n=48) showed positive VEGF expression and 9 cases (18.7%) showed negative expression. On the other hand, proportion of negative VEGF expression was lower (18.5%) among patients with low grade carcinoma. This indicates that with progression in tumor grade, the rate of VEGF expression significantly increases; which was similar to Rahmani et al. & Yang et al. (29,31).

Our study also evaluated the association between VEGF expression & muscle-invasiveness of the urinary bladder carcinomas & revealed that 55 (85.9%) NMIBC (n=67) cases were positive for VEGF expression. Whereas, only 9 (14.1%) MIBC (n=12) cases showed positive VEGF expression & 3 (20.0%) cases were negative for VEGF expression. This is in concordance with the study done by Kopparapu et al. in 2013, showing that VEGF expression was significantly higher in NMIBC as compared to MIBC(45). Also, in 2019, Özveren and Türkeri observed that VEGF positive expression was higher in patients with NMIBC (45%) and in high grade tumors (44%). However, the study conducted by Yang et al. (46) observed significantly high expression of VEGF in MIBC & its association with poor prognosis.

TABLE 17: VEGF EXPRESSION IN VARIOUS STUDIES

AUTHOR	CASES	VEGF EXPRESSION (%)	ASSOCIATED STAGE & GRADE
Anika Sadaf et al(23)	49	-75.5 -100	-MIBC -NMIBC
Vishakha Behl et al(28)	50	-55	-HGUC
Arshad Rahmani et al(30)	125	-43.2	-HGUC
Ching-ChiangYang et al(33)	161	-29.0 -73.9	-NMIBC -MIBC
Yii-Her Chou et al(41)	301	-12 -28.5 -29.2 -26.6 -4.7	-pTa/pTis -pT1 -pT2 -pT3 -pT4
Present study	79	-85.9 -14.1	-NMIBC -MIBC

None of the studies have compared or correlated the expression of FGFR3 & VEGF together in urothelial carcinomas. In our study it was observed that both these immunohistochemical markers were highly expressed in non-muscle invasive urothelial cancers i.e. stage pTa/pT1, as compared to muscle invasive urothelial cancers pT2.

Summary & conclusion

The present study was done at All India Institute of Medical Sciences, Jodhpur. Herein we assessed the expression of Fibroblast Growth Factor Receptor 3 (FGFR3) & Vascular Endothelial Growth Factor (VEGF) in malignant tumors of the urothelial tract received in the Department of Pathology & Lab Medicine by immunohistochemistry and correlated its expression with various clinicopathological parameters. The antibodies for FGFR3 & VEGF were applied manually in all 79 cases of urothelial carcinomas and the interpretation of the IHC was done by semi-quantitative (Q score) scoring system.

- Among 79 cases of urothelial carcinoma, FGFR3 positive expression by immunohistochemistry was seen in 86.5% of the non-muscle invasive urothelial carcinomas & 13.5% of muscle invasive urothelial carcinomas.
- Positive expression of VEGF was observed in 85.9% of non-muscle invasive urothelial carcinomas & 14.1% of muscle invasive urothelial carcinomas.
- Mean age for development of urothelial carcinoma was 61 years
- Males were more affected than females with a male to female ratio of 4:1
- It was observed that both FGFR3 & VEGF were highly expressed in non-muscle invasive urothelial carcinomas as compared to muscle invasive urothelial carcinomas

A limitation of present study is small sample size due to exclusion of cases that had received any chemo or radio therapy for urothelial carcinoma.

In conclusion, though the present study showed expression of FGFR3 & VEGF in predominately non- muscle-invasive urothelial carcinomas. Further studies with a bigger sample size are required to establish correlation between these two markers and prognostic factors of urothelial carcinoma especially in the Indian cohort. In the current era of personalised cancer treatment and targeted therapy, role of bio markers like FGFR3 & VEGF is becoming more relevant in cancer management. Immunotherapy is gradually revolutionising the bladder cancer management hence, the present study underscores the need for more clinical trials to shed light on the role of FGFR3 & VEGF in bladder cancer management & treatment.

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48. Key Statistics for Bladder Cancer

ANNEXURES

1. IEC certificate
2. **Informed Consent form – English – 9A**
3. **Informed Consent form – Hindi – 9B**
4. **Patient Information Sheet – English – 9C**
5. Patient Information Sheet – Hindi – 9D
6. Performa
7. Master chart

Ethical Justification

- Informed written consent was taken from all study subjects. No pressure orcoercion was exerted on subjects for participation in study.
- Confidentiality and privacy were maintained at all stages.
- Enrolment in the study posed no risk to the patient and did not increase the cost of the treatment
- Informed written consent was taken from all the patients as per the attached Performa.

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

No. AIIMS/IEC/2021/3345 Date: 12/03/2021

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3345**

Project title: **"Expression of fibroblast growth factor receptor 3 (FGFR3) and vascular endothelial growth factor (VEGF) in malignant tumors of the urothelial tract"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr. Apurva Arora**
Guide:	**Dr. Aasma Nalwa**
Co-Guide:	**Dr. Poonam Abhay Elhence, Dr. Meenakshi Rao & Dr. Gautam Ram Chaudhary**

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. Pragati 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

All India Institute of Medical Sciences
Jodhpur, Rajasthan
Informed Consent Form

Title of the project: **Expression of Fibroblast growth factor receptor 3 (FGFR3) and Vascular endothelial growth factor (VEGF) In Malignant Tumors of the Urothelial tract**

Name of the Principal Investigator : Dr. Apurva arora Tel. No. 9416110820

Patient / Volunteer Identification No.:_____

I, _____ S/o or D/o _____ R/o

_____

give my full, free, voluntary consent to be a part of the study "_____", the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individual from _____ - (Company Name) or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____ _____

Place: _____ Signature/Left thumb impression

Date: _____ _____

This to certify that the above consent has been obtained in my presence.

Place: _____ Signature of Principal Investigator

Witness 1

Signature _____

Name: _____

Address: _____

Witness 2

Signature_____

Name: _____

Address: _____

All India Institute of Medical Sciences
Jodhpur, Rajasthan
Informed consent form (Hindi)

थीसिस / निबंधकाशीर्षक: **यूरोटेलियल ट्रैक्ट के घातक ट्यूमर में फाइब्रोब्लास्ट ग्रोथ फैक्टर रिसेप्टर 3 (एफजीएफआर3) और वैस्कुलर एंडोथेलियल ग्रोथ फैक्टर (VEGF) की अभिव्यक्ति**

पीजी छात्र का नाम: डॉ अपूर्वा अरोड़ा न. : 9416110820

रोगी / स्वयं सेवक पहचान संख्या: _____

मैं,_____ S/o or D/o _____
R/o
_____

अध्ययन "" _____

का एक भाग बनने के लिए मेरी पूर्ण, स्वतंत्र ,स्वैच्छिक सहमति दें,जिसकी प्रक्रिया और प्रकृति मुझे अपनी पूरी संतुष्टि के लिए अपनी भाषा में समझाई गई है। मैं पुष्टि करता हूं कि मुझे प्रश्न पूछने का अवसर मिला है।

मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मुझे किसी भी कारण दिए बिना किसी भी समय अध्ययन से बाहर निकलने के मेरे अधिकार की जानकारी है।

मैं समझता हूं कि मेरे और मेरे मेडिकल रिकॉर्ड के बारे में एकत्रित की गई जानकारी

को_____ (कंपनीनाम) या विनियामक प्राधिकरणों से जिम्मेदार व्यक्ति द्वारा देखा जा सकता है। मैं इन व्यक्तियों को अपने अभिलेखों तक पहुंच के लिए अनुमति देता हूं।

Date : _____

जगह: _____ हस्ताक्षर/ बाएं अंगूठे का छाप_____

यह प्रमाणित करने के लिए कि मेरी उपस्थिति में उपरोक्त सहमति प्राप्त की गई हैI

तारीख : _____ जगह: _____

पीजी छात्र के हस्ताक्षर_____

गवाह1 : _____ हस्ताक्षर: _____ तारीख : _____	गवाह2: _____ हस्ताक्षर: _____ तारीख : _____

PATIENT INFORMATION SHEET (English)

1. Risks to the patients: No interventions or life-threatening procedure will be done.

2. Confidentiality: Your participation will be kept confidential. Your medical records will be treated with confidentiality and will be revealed only to doctors/ scientists involved in this study. The results of this study may be published in a scientific journal, but you will not be identified by name.

3. Provision of free treatment for research related injury. Not applicable.

4. Compensation of subjects for disability or death resulting from such injury: Not Applicable

5. Freedom of individual to participate and to withdraw from research at any time without penalty or loss of benefits to which the subject would otherwise be entitled.

6. Your participation in the study is optional and voluntary.

7. The copy of the results of the investigations performed will be provided to you or your record.

8. You can withdraw from the project at any time, and this will not affect your subsequent medical treatment or relationship with the treating physician.

9. Any additional expense for the project, other than your regular expenses, will not be charged from you.

रोगी सूचना पत्रक

1. रोगियों के लिए जो खिम: कोई हस्तक्षेप या जीवन-धम की प्रक्रिया नहीं की जाएगी।

2. गोपनीयता: आपकी भागीदारी को गोपनीय रखाजाएगा। आपके मेडिकल रिकॉर्ड को गोपनीयता के साथ इलाज किया जाएगा और केवल इस अध्ययन में शामिल डॉक्टरों / वैज्ञानिकों को पता चलेगा। इस अध्ययन के परिणाम एक वैज्ञानिक पत्रिका में प्रकाशित हो सकते हैं,लेकिन आपको नाम से पहचाना नहीं जाएगा।

3. अनुसंधान संबंधी चोट के लिए नि:शुल्क उपचार कीव्यवस्था: लागू नहीं।

4. ऐसी चोट से उत्पन्न विकलांगता या मृत्यु के लिए विषयों का मुआवजा: लागू नहीं हैI

5. किसी भी समय दंड या लाभों के नुकसान के बिना किसी भी समय भाग लेने के लिए व्यक्ति को स्वतंत्रता लेने और अनुसंधान से वापस लेने के लिए स्वतंत्रता,जिसके तहत विषय अन्यथा हकदार होगाI

6. आपको जुर्माना या लाभ के नुकसान के बिना किसी भी समय भाग लेने और अनुसंधान से वापस लेने की पूरी आजादी है,जिस पर आप अन्यथा हकदार होंगे।

7. अध्ययन में आपकी भागी दारी वैकल्पिक और स्वैच्छिक है।

8. प्रदर्शन की जांच की परिणामों की प्रति आपके रिकॉर्ड के लिए आपको उपलब्ध कराई जाएगी।

9. आप किसी भी समय परियोजना से वापस ले सकते हैं ,और यह आपके बाद के चिकित्सा उपचार या उपचार चिकित्सक के साथ संबंध को प्रभावित नहीं करेगा।

10. परियोजना के लिए कोई भी अतिरिक्तव्यय,आपके नियमित खर्चों के अलावा,आप से शुल्क नहीं लिया जाएगा ।

All India Institute of Medical Sciences (AIIMS), Jodhpur
Department of Pathology
PROFORMA

Date:

Name

Age: Sex: I.D:

Address:

Relevant clinical History:

Family history of malignancy:

Histological diagnosis (with histopathological stage if available):

Requested information for optimal patient care:

(1) Known/Previous malignancy: ☐ 2) Clinical tumor staging information: ☐

(3) Immunocompromised: ☐ (4) Chemotherapy: ☐ (5) Radiotherapy: ☐

(6) Immunohistochemistry:

- **FGFR 3** - Positive ☐ Negative ☐

If positive:

Q score = intensity x percentage staining

(a) Intensity of stain

No stain: 0 ☐

Faint/detectable stain in some or all tumor cells: 1+ ☐

Week stain (extensive positivity) : 2+ ☐

Strong Positivity: 3+ ☐

(b) Percentage score – 0-3

- **VEGF-** Positive ☐ Negative ☐

(Scored according to the intensity and percentage of tumor cells showing cytoplasmic & membranous positivity.)

Q score = intensity x percentage staining

(a) Intensity of stain

No stain: 0 ☐

Faint/detectable stain in some or all tumor cells: 1+ ☐

Week stain (extensive positivity) : 2+ ☐

Strong Positivity: 3+ ☐

(b) Percentage score – 0-3

Master Chart

Histo No.	SEX	Age	SPECIMEN	DIAGNOSIS	INVASION	FGFR3 INTERPRETATION		RESULT	VEGF INTERPRETATION		RESULT
H/3226/16	M	65	T	HG	I	0	0	N	2x2	4	P
H/3282/16	F	89	T	HG	I	1x2	2	P	0	0	N
H/2101/16	M	87	T	LG	N	2x2	4	P	0	0	N
H/2214/16	M	61	T	HG	N	3x1	3	P	0	0	N
H/2424/16	M	68	T	HG	N	3x4	12	P	0	0	N
H/2589/16	M	56	T	HG	D	2x3	6	P	0	0	N
H/0443/17	M	55	T	LG	N	0	0	N	2x3	6	P
H/0555/17	F	84	T	SCC	N	2x3	6	P	0	0	N
H/0687/17	M	59	T	LG	N	2x4	8	P	1x1	1	N
H/1361/17	M	79	T	HG	I	3x4	12	P	3x4	12	P
H/1608/17	M	61	T	HG	N	3x4	12	P	3x4	12	P
H/3059/17	M	65	T	LG	N	3x4	12	P	2x2	4	P
H/3074/17	M	60	T	HG	N	1x1	1	N	3x4	12	P
H/3075/17	M	73	T	LG	I	2x4	8	P	3x4	12	P
H/3464/17	M	63	RCP	HG	D	1x1	1	N	2x1	2	P
H/3602/17	M	75	RCP	HG	D	1x1	1	N	3x4	12	P
H/3740/17	F	77	NEPHROURETERECTOMY	LG	N	1x1	1	N	0	0	N
H/0810/18	M	45	T	HG	N	3x3	9	P	0	0	N
H/0904/18	M	77	T	LG	N	1x4	4	P	1x1	1	N
H/1175/18	M	55	T	LG	N	3x4	12	P	3x4	12	P
H/5027/18	M	76	T	HG	I	2x3	6	P	2x3	6	P
H/3782/18	M	46	T	LG	N	0	0	N	3x4	12	P
H/5780/18	F	56	T	HG	I	2x2	4	P	3x4	12	P
H/5827/18	M	52	T	HG	I	1X4	4	P	3x4	12	P
H/6218/18	M	35	T	LG	N	1x1	1	N	3x4	12	P
H/7195/18	F	63	T	SCC	I	0	0	N	3x4	12	P
H/7691/18	M	72	T	HG	N	0	0	N	3x4	12	P
H/7040/18	M	49	T	HG	I	2x4	8	P	0	0	N
H/99/19	M	87	T	HG	I	0	0	N	3x4	12	P
H/313/19	M	87	T	HG	I	0	0	N	3x4	12	P
H/1000/19	M	58	T	LG	N	0	0	N	2x3	6	P
H/1557/19	M	71	T	HG	D	0	0	N	0	0	N
H/1560/19	M	59	T	SCC	I	0	0	N	3x4	12	P
H/3460/19	F	46	T	HG	N	0	0	N	2x3	6	P
H/3757/19	M	49	T	HG	N	1x1	1	N	3x3	9	P
H/3879/19	M	48	T	LG	I	0	0	N	3x4	12	P
H/4455/19	M	61	T	LG	N	2x2	4	P	3x4	12	P
H/4952/20	M	52	T	LG	I	1x1	1	N	2x2	4	P
H/4547 /20	M	60	T	HG	D	0	0	N	3x4	12	P
H/4470/20	F	44	T	LG	I	1x2	2	P	2x3	6	P
H/7556/21	F	20	T	HG	D	0	0	N	0	0	N
H/6740/21	F	38	T	HG	I	3x4	12	P	2x4	8	P
H/6657/21	M	57	T	HG	I	3x2	6	P	3x4	12	P
H/5760/21	M	70	T	LG	N	3x4	12	P	3x4	12	P
H/4873/21	M	69	T	HG	I	3x4	12	P	3x4	12	P
H/3393/21	M	69	T	HG	I	1x1	1	N	3x4	12	P
H/3105/21	M	64	T	HG	I	3x4	12	P	3x4	12	P
H/2382/21	M	50	RCP	LG	I	2x3	6	P	2x3	6	P
H/2538/21	F	72	T	HG	D	3x4	12	P	3x4	12	P
H/2189/21	M	52	T	HG	D	3x4	12	P	3x4	12	P
H/2156/21	M	58	T	HG	D	3x4	12	P	3x4	12	P
H/2108/21	M	69	T	HG	D	3x4	12	P	3x4	12	P
H/0125/21	M	78	T	LG	I	0	0	N	2x2	4	P
H/0025/21	M	44	T	HG	N	1x1	1	N	2x4	8	P
H/1348/22	F	43	T	LG	N	3x4	12	P	3x4	12	P
H/1610/22	M	57	T	HG	I	3x4	12	P	3x4	12	P
H/2021/22	M	45	T	LG	N	2x4	8	P	3x4	12	P
H/2171/22	M	45	RCP	SC-NEC	I	0	0	N	3x4	12	P
H/3227/22	M	72	T	LG	N	3x4	12	P	3x4	12	P
H/5164/22	M	51	RCP	HG	N	3x4	12	P	2x1	2	P
H/3818 /22	M	73	T	HG	N	3x4	12	P	3x4	12	P
H/4120/22	M	45	T	HG	D	3x4	12	P	3x4	12	P
H/4215 /22	M	47	T	HG	I	3x2	6	P	3x4	12	P
H/4348 /22	M	77	T	HG	I	0	0	N	3x4	12	P
H/4652 /22	F	60	T	LG	N	3x4	12	P	1x2	2	P
H/5050/22	F	54	T	HG	I	3x4	12	P	3x3	9	P
H/5052/22	M	50	T	LG	N	3x4	12	P	3x3	9	P
H/5216 /22	M	54	T	HG	I	2x4	8	P	3x4	12	P
H/5248/22	M	57	T	HG	I	1x1	1	N	2x2	4	P
H/5613/22	F	67	T	HG	N	3x4	12	P	3x4	12	P
H/5752 /22	M	68	T	LG	N	3x2	6	P	2x3	6	P
H/5754 /22	M	60	T	HG	I	3x4	12	P	3x3	9	P
H/5811/22	M	53	T	LG	N	3x4	12	P	1x1	1	N
H/6234/22	M	72	T	HG	I	3x4	12	P	0	0	N
H/6377/22	M	67	RCP	HG	D	2x3	6	P	2x2	4	P
H/6501/22	M	52	T	LG	N	3x4	12	P	3x4	12	P
H/6750/22	F	60	T	LG	N	3x3	9	P	3x3	9	P
H/6752/22	M	78	T	HG	I	2x3	6	P	3x3	9	P
H/7271/22	F	78	T	HG	N	3x4	12	P	3x4	12	P