"EVALUATION OF ROLE OF GATA3 EXPRESSION IN DIFFERENTIATING UROTHELIAL CARCINOMAS FROM ADENOCARCINOMAS OF PROSTATE"

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LIST OF ABBREVIATIONS USED

LGUC	-	Low grade urothelial carcinoma
HGUC	-	High grade urothelial carcinoma
CIS	-	Carcinoma in situ
H&E	-	Haematoxylin and eosin
IHC	-	Immunohistochemistry
HMWCK	-	High molecular weight cytokeratin
FGFR3	-	Fibroblast growth factor receptor 3
PUNLMP	-	Papillary urothelial neoplasm of low malignant potential
LVI	-	Lymphovascular invasion
PNI	-	Perineural invasion
TUR	-	Transurethral resection
BCG	-	Bacille Calmette Guerin
PSA	-	Prostate specific antigen
PSAP	-	Prostatic specific acid phosphatase
PSMA	-	Prostate specific membrane antigen
LH-RH	-	Luteinizing hormone–releasing hormone
HIFU	-	High-intensity focused ultrasound
TNM	-	Tumour, nodes and metastases
WHO	-	World Health Organisation
ISUP	-	International Society of Urological Pathology
DPX	-	Dibutylphthalate Polystyrene Xylene
TILs	-	Tumour infiltrating lymphocytes
HPF	-	High power field

ABSTRACT

"EVALUATION OF ROLE OF GATA3 EXPRESSION IN DIFFERENTIATING UROTHELIAL CARCINOMAS FROM ADENOCARCINOMAS OF PROSTATE"

Background and objectives: Bladder cancer ranks 10th among the most frequently diagnosed cancers and are more common among men while prostate cancer ranks 2nd among the most frequently diagnosed cancer in men worldwide. Sometimes on routine hematoxylin and eosin (H&E) stain, it becomes difficult to differentiate between urothelial and prostate adenocarcinomas as they have similar morphological features in advanced stages. They also differ in their treatment modalities, staging and prognosis. The purpose of this study is to evaluate the role of GATA3 expression in differentiating urothelial carcinomas from adenocarcinomas of prostate and to correlate GATA3 positivity score with clinicopathological parameters.

Methods: The present study was a prospective as well as retrospective study which included 40 cases of urothelial carcinoma and 40 cases of prostate adenocarcinoma from January 2018 to December 2020. The clinical details pertaining to the patients under study were obtained from the medical records department of the hospital. The gross and histopathology reports were collected from the report forms archived in the pathology department. The blocks embedded in paraffin were retrieved; slides were made from these blocks and stained for H&E and GATA3 marker. Statistical analysis was done applying chi square test. The probability value (p) of ≤ 0.05 was considered statistically significant.

Results: The GATA3 marker stained positive for 30/40 (75%) cases of urothelial carcinoma. The GATA 3 IHC marker stained negative for all 40 cases of prostate adenocarcinoma. Out of the 10 negative cases of urothelial carcinoma, 7 cases belong to high grade and 3 cases belong to low grade.

Conclusion: The results of the study showed that GATA3 marker is an important marker that can be incorporated into the IHC panel for differentiating urothelial carcinomas from prostate adenocarcinomas. No statistically significant association between the clinicopathological parameters including tumour grade with GATA3 expression was observed.

Key words: GATA3 marker, immunohistochemistry, urothelial carcinomas, prostate adenocarcinomas.

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INTRODUCTION

Bladder cancer ranks 10th among the most frequently diagnosed cancers and are more common among men when compared to women while prostate cancer ranks 2nd among the most frequently diagnosed cancer in men worldwide as per the GLOBOCAN 2018 (An online data base that provides the world cancer statistics and estimates).[1] In India, bladder cancer ranks 18th and prostate cancer ranks 16th in terms of incidence in the year 2018. [2] Also, there is an increase in trend observed for 5 year prevalence rates in India for bladder and the prostate cancers between the years 2018 and 2020. For bladder cancers, it has risen from 3.21% to 3.57% and for prostate cancer, it has risen from 6.78% to 9.47% respectively.[2, 3]

Bladder cancers mainly arise from the epithelium of bladder and are usually urothelial carcinomas.[4] Urothelial carcinomas may range from papillary to flat in morphology. These carcinomas can be low grade or high grade and invasive or non-invasive in nature.[5] Urothelial carcinomas can sometimes be aggressive or can have multiple recurrences after local resection of the tumour.[6]

Prostatic adenocarcinoma is an important differential diagnosis to be ruled out while assessing a case of urothelial carcinoma. The prostate maybe involved by infiltrating urothelial carcinoma either by direct invasion into prostate stroma or by intraductal extension with or without the invasion into prostate stroma. Also, bladder is sometimes involved by prostate adenocarcinoma either by metastasis or through direct extension to the bladder. Prostate is a second common primary site for secondary bladder carcinomas.[7]

It is pathologically important to distinguish a high grade prostate adenocarcinoma extending in to urinary bladder from a high grade urothelial carcinoma infiltrating the prostate as these cancers differ in their treatment modalities, staging and prognosis.[6] Urothelial carcinomas in advanced stages may be treated with chemotherapy while prostate adenocarcinomas are usually treated using anti-androgen hormone therapy.[8]

Sometimes on routine hematoxylin and eosin (H&E) stain, it becomes difficult to differentiate between urothelial and prostate adenocarcinomas as in the advanced stages of these carcinomas, they have similar morphological characteristics and clinical manifestations. In such instances, it becomes necessary to do immunohistochemistry to differentiate between the two cancers. [4]

GATA3 is a type of transcription factor having zinc finger domain. It is encoded by GATA3 gene located on chromosome 10p14.[9] The carboxyl end contains 2 zinc fingers. It belongs to the tumour genes family with tumour suppressor function. It helps in promoting proliferation and differentiation in numerous cells and tissues like luminal epithelial cells of mammary gland, adipose tissue, thymocytes, kidney, T lymphocytes, sympathetic nervous system and hair follicles in skin.[10,11] It has now been recognized as an immunohistochemical (IHC) marker for urothelial carcinomas helping to differentiate it from prostatic adenocarcinomas.

However, there are only few studies done in India to understand the utility of GATA3 IHC marker for this purpose. Therefore, this study will be done to understand the utility of GATA3 IHC marker in differentiating urothelial carcinomas from prostatic adenocarcinomas and to correlate clinicopathological parameters with GATA3 expression.

OBJECTIVES

1. To evaluate the role of GATA3 expression in differentiating urothelial carcinomas from adenocarcinomas of prostate.
2. To correlate GATA3 positivity score with clinicopathological parameters.

REVIEW OF LITERATURE

URINARY BLADDER

EMBRYOLOGY:

The bladder has its origin from vesicourethral canal, a portion of urogenital sinus and lies above the mesonephric duct opening. It has a narrow lower part and a wide upper part. The wide upper part receives the opening of the allantois at its apex.[12] The dilated upper part of vesicourethral canal along with the proximal part of allantois forms the urinary bladder while the narrow lower part forms the primitive urethra. The bladder trigone has a mesodermal origin and formed by absorption of mesonephric ducts along with ureteric buds into the posterior wall of vesicourethral canal.[13] Upper portion of vesicourethral canal is from endoderm and forms the epithelium of bladder and the absorbed part of mesonephric ducts is mesodermal in origin and forms epithelium of trigone of bladder. The splanchnopleuric mesoderm forms muscular as well as serous walls of bladder. The allantois later atrophies in to a fibrous band in the post-natal life to form the median umbilical ligament that extends from the apex of bladder to umbilicus.[12]

ANATOMY:

The bladder is the anteriormost muscular organ inside pelvic cavity of humans and vertebrates. It acts as a temporary storage for urine before its release from the body. The urine reaches the bladder from the kidney via the 2 ureters and is later removed from the bladder through the urethra.[14]

The detrusor muscle forms the major portion of bladder wall and consists of spiral and circular bundles of smooth muscle fibres. This muscle helps in the bladder contraction. An empty bladder completely lies within pelvic cavity and arises in to the abdominal cavity once filled with urine.[16]

An empty bladder forms a tetrahedral shape. It consists of: [14]

a) Apex: It is points towards the upper portion of pubic symphysis.

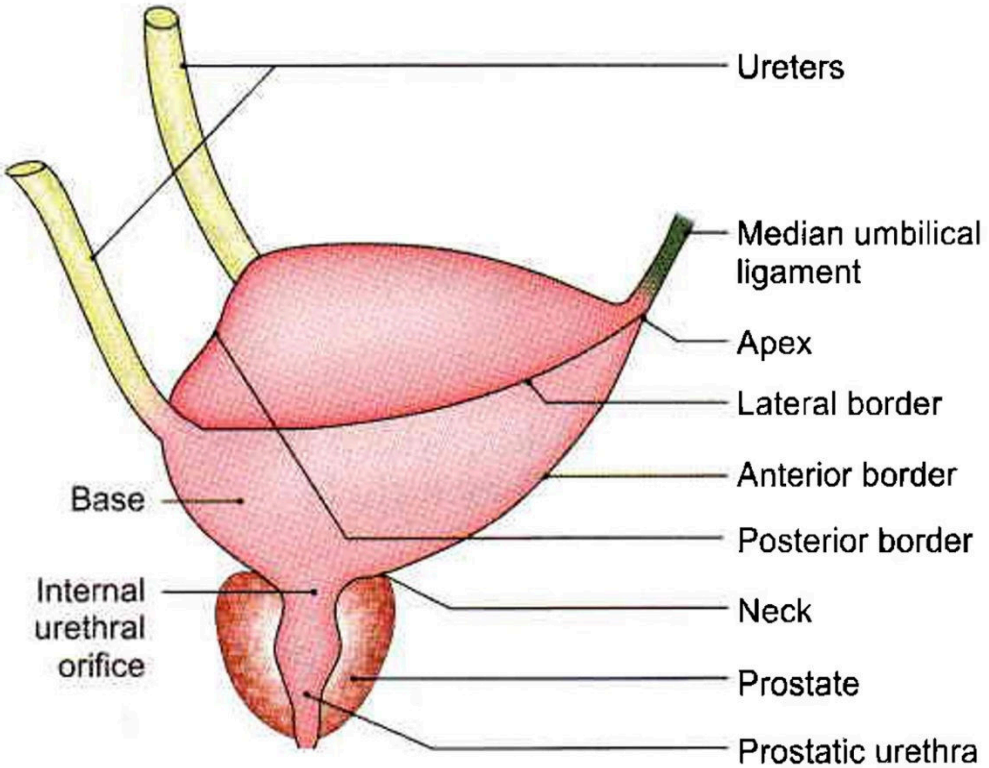
b) Base or fundus: It is points backwards and faces posteroinferiorly.

c) Neck: It is the fixed part of bladder and is located inferiorly.

d) Three surfaces: 2 inferolateral surfaces (right and left) and a superior surface.

e) Four borders: 1 anterior border, 1 posterior border, 2 lateral borders.

Figure 1: External features of bladder [14]



(**Image taken from:** *Chaurasia BD. BD chaurasia's human anatomy regional and applied dissection and clinical: Vol. 2: Lower limb abdomen and pelvis. 6th ed. New Delhi, India: CBS Publishers & Distributors; 2013*)

When bladder is full, it forms an ovoid shape. It consists of: [14]

a) Apex: It points towards umbilicus and connects to it with the help of median umbilical ligament
b) Neck: It points downwards.
c) 2 surfaces: Anterior and posterior.

In females, the base of bladder is near to cervix and vagina. In males, the top portion of base as well as rectum and intestine are separated by rectovesical pouch while the below part of base is in relation with the end part of vas deferens as well as seminal vesicles.[15]

The neck region of bladder lies behind the public symphysis above the pelvic outlet and consists of internal urethral orifice. The bladder in males has a peritoneal covering on it superior surface which is related to terminal part of ileum and the sigmoid colon. In females, the peritoneum covers major portion of the superior surface except for the part close to the posterior surface that lies in relation to supravaginal part of the uterine cervix.[16]

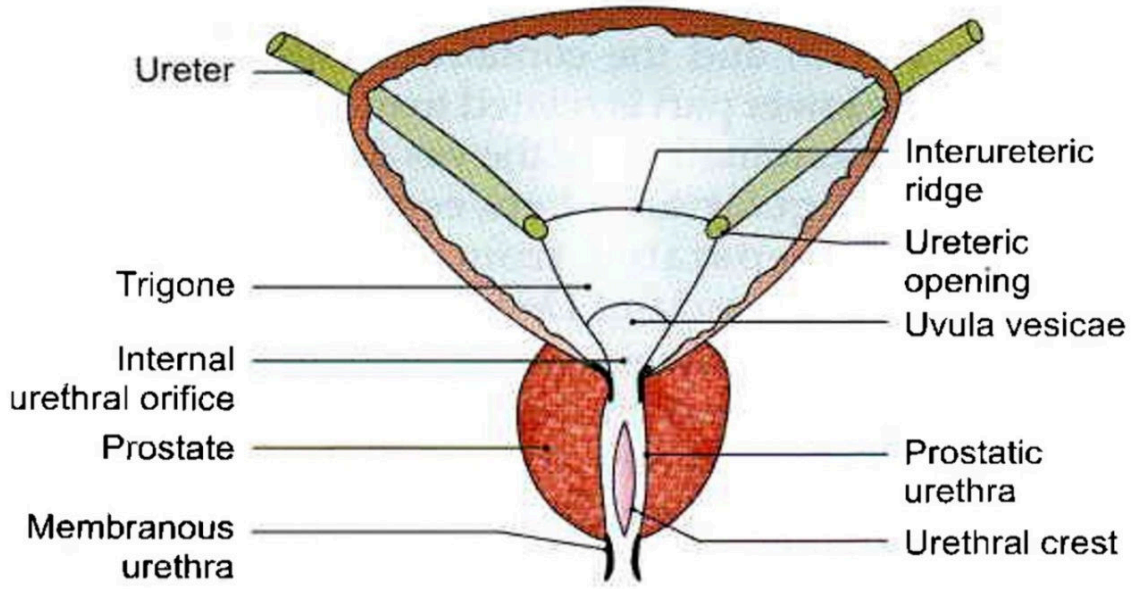
In the bladder, the inferolateral surfaces do not have peritoneal covering and lies in relation to puboprostatic ligaments, levator ani and obturator internus. In females, puboprostatic ligaments are replaced by pubovesical ligaments.[15]

INTERIOR OF URINARY BLADDER:

Bladder has a smooth mucosal lining at the base which is firmly attached to smooth muscle coat below. The remaining portion of mucosa form folds and is attached with the underlying layer loosely.[15]

The interior of bladder has a triangular smooth area known as trigone of bladder and it lies in between the ureter and urethral openings. In an empty bladder, the two openings of the ureter are around 2.5 cm apart and when bladder distends, they are around 5 cm apart. Internal urethral orifice opens in to urethra at the trigone apex. An elevated portion on the trigone of bladder behind urethral orifice is called as uvula vesicae and is produced by the prostate's median lobe. The trigone base is made by interureteric ridge.[14]

Figure 2: Interior of urinary bladder [14]



(**Image taken from:** *Chaurasia BD. BD Chaurasia's human anatomy regional and applied dissection and clinical: Vol. 2: Lower limb abdomen and pelvis. 6th ed. New Delhi, India: CBS Publishers & Distributors; 2013*)

LIGAMENTS OF BLADDER:

TRUE LIGAMENTS: True ligaments are the condensed fascia of pelvis at the neck and base of bladder. They include: [16]

1. The lateral true ligaments which lie on either sides of bladder and connect these sides to tendinous arch of pelvic fascia.
2. The medial side puboprostatic ligaments that extends from behind pubic bone to prostate sheath.
3. The lateral side puboprostatic ligaments that extend between tendinous arch of pelvic fascia and upper part of prostatic sheath. The puboprostatic ligaments are replaced by pubovesical ligaments in female.
4. The posterior ligaments extend on either side from bladder base up to pelvic wall.
5. Median umbilical ligament

FALSE LIGAMENTS: False ligaments are basically peritoneal folds and do not support the bladder. They constitute: [16]

1. Lateral false ligament
2. Posterior false ligament
3. Median and medial umbilical folds

CAPACITY OF THE BLADDER:

The mean capacity of the bladder is 220 ml (120 – 320 ml). There is a feeling to micturate beyond 220 ml. When it fills to about 250 - 300ml, the bladder is emptied. A person can tolerate up to a maximum of 500 ml beyond which it causes a referred pain at anterior abdominal wall (lower portion), perineum as well as penis.[14]

ARTERIAL SUPPLY OF BLADDER:

1. Superior and inferior vesical artery (In females, uterine and vaginal artery instead of inferior vesical artery).
2. Obturator and inferior gluteal artery [16]

VENOUS DRAINAGE OF BLADDER:

Venous drainage is by vesical venous plexus that lies on inferolateral surfaces of bladder and they drain into the internal iliac veins.[16]

LYMPHATIC DRAINAGE OF BLADDER:

The lymphatics usually end in the external iliac nodes and a few into the internal iliac nodes or lateral aortic nodes.[16]

NERVE SUPPLY OF BLADDER:

The vesical plexus of nerves supply bladder which arise from inferior hypogastric plexus. They have both sympathetic and parasympathetic components: [14]

1) The parasympathetic efferent fibres, S2, S3, S4 are motor to the detrusor muscle and helps in normal micturition.
2) The sympathetic efferent fibres from the T11 to L2 nerves. It has motor function on the preprostatic sphincter and inhibits the action of detrusor muscle.
3) The somatic pudendal nerve arises from S2 to S4 nerves. They supply the urethral sphincter in the urethral wall
4) Pain sensations from distension or spasm bladder are carried mainly by the parasympathetic nerves and to a lesser extent by sympathetic nerves.
5) Pain from the bladder is carried via lateral spinothalamic tract of spinal cord.
6) Bladder distension awareness is via the posterior column of spinal cord.

HISTOLOGY OF BLADDER:-

Histologically, the bladder wall constitutes 4 layers:

Epithelium (urothelium):

The bladder has a transitional epithelium. The basal layer has cells which are cuboidal or columnar in shape. Above this layer, there are numerous layers of cells which are polyhedral in shape. The superficial or the luminal layer is formed by large cells which have eosinophilic abundant cytoplasm with a free rounded surface and hence are called as umbrella cells. The thickness and shape of the cells forming the urothelium varies depending on the distension of bladder. The urothelial lining is thicker and consists of 6- 7 layers of cells when empty. The thickness reduces to about 3-4 layers when distended. Also on distension, the basal layer consists of mainly cuboidal cells and the cells in the superficial layer becomes elongated and flattened with inconspicuous umbrella cells. The urothelium and lamina propria is separated by thin layer of basement membrane.[17]

Lamina propria:

This layer contains abundant connective tissue within which lies the vascular channels, lymphatic channels, nerve fibres and elastic fibres. This layer is thin at the neck and trigone of bladder. They also contain some amount of smooth muscles or muscularis mucosae.[15] The thickness of lamina propria is variable depending on whether bladder is empty or distended. Some amount of fat may be seen.[17]

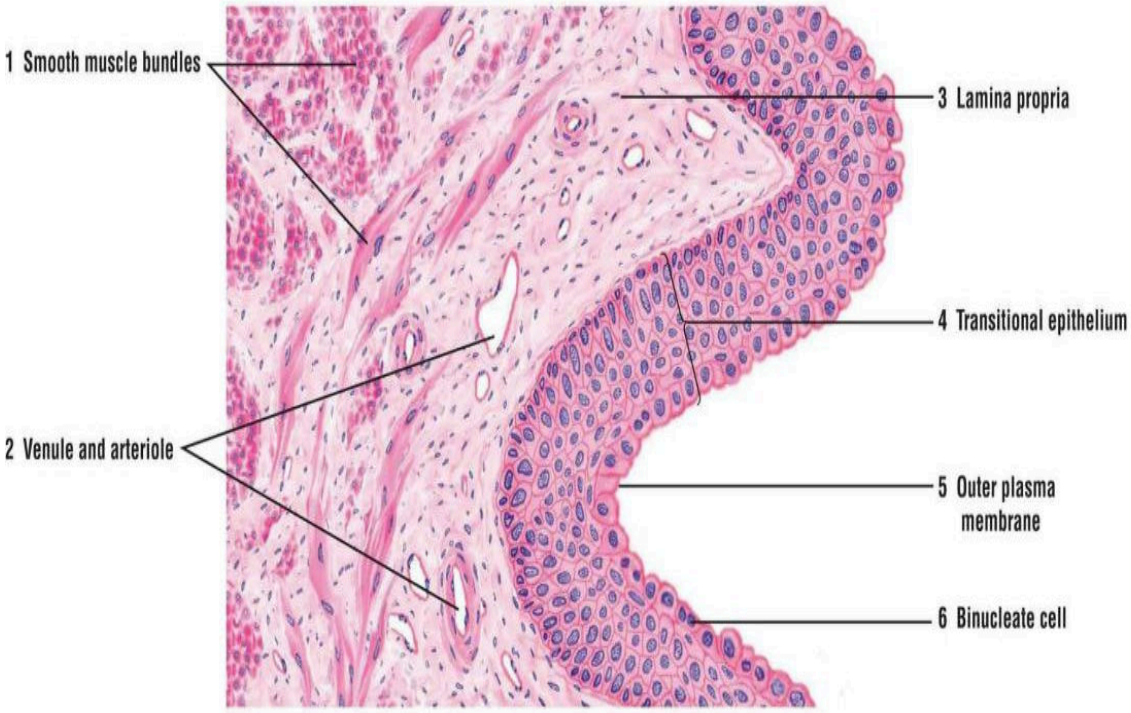
Muscularis propria:

This layer has loosely arranged internal as well as an external longitudinal layer along with a prominent middle circular muscle layer.[18] In males, this layer is continuous with prostatic fibromuscular tissue at neck of bladder. In females, the muscularis propria continues with the muscle fibers of urethral wall. Some amount of fat may be seen.[17]

Adventitia or serosa:

Outermost layer of bladder is a connective tissue adventitia. The bladder superior surface has a serosal covering of the pelvic peritoneum and the inferior surface has connective tissue adventitia that merges with the connective tissue of adjacent structures. The mesothelium covers the serosa.[17]

Figure 3. Histology of normal urinary bladder [17]



(**Image taken from:** *Eroschenko VP. Atlas of histology with functional correlations. 13th ed. Philadelphia, PA: Lippincott Williams and Wilkins; 2016*)

BLADDER CARCINOMA

Bladder cancer ranks 10th in most frequent diagnosed cancers worldwide.[1] Its incidence in developed countries is higher than in developing countries and also in urban than in rural areas. The incidence is high among 60-70 years of age. Bladder cancers are more common in the men when compared to women. Majority of urinary bladder carcinoma has epithelial origin and are usually urothelial carcinoma.[19]

ETIOLOGY AND PATHOGENESIS

The bladder cancer develops due to have a variety of genetic and environmental factors implicated in its causation. Some of the important environmental factors that results in bladder carcinoma includes the following: [20]

1. Cigarette smoking is an important factor that increases the risk of bladder cancers depending on the type and duration of tobacco use.
2. Prolonged exposure to aryl amines in the industries can result in bladder cancer in 15-40 years after the first exposure.
3. Long term exposure to aniline dyes (particularly benzidine and β-naphthylamine), auramines, phenacetin, and cyclophosphamide can contribute to cancer formation.
4. Schistosoma haematobium infection is an established risk factor as these organisms cause a brisk chronic inflammation when their ova gets deposited on the bladder wall resulting in squamous metaplasia and dysplasia of the mucosa and sometimes even neoplasia.
5. Irradiation usually done for other pelvic malignancies can also cause bladder cancer after many years.
6. Use of analgesics for long term can cause bladder cancer

Some of the genetic alterations can result in bladder cancers. These include: [20]

1. Gain-of-function mutations in Fibroblast growth factor receptor 3 (FGFR3) are seen usually in non-invasive low grade papillary carcinoma. They cause activation of the FGFR3 receptor tyrosine kinase.
2. Activating mutations in the HRAS oncogene have been noted in non-invasive, low grade tumours.

3. In high grade and muscle invasive tumours, there is loss of function mutations in the RB and TP53 tumour suppressor genes.
4. Genetic material may be lost on chromosome 9 leading to monosomy or deletions of 9 p or 9q are in seen in non-invasive flat tumours or superficial non-invasive papillary tumours. The 9p deletions affects tumour suppressor gene like CDKN2A that encodes cyclin dependent kinase inhibitor p16/INK4a and a protein ARF that increase p53 function.

CLINICAL PRESENTATION OF BLADDER CARCINOMA:

1. Hematuria (80-90% cases) – Generally gross painless hematuria and even hematuria microscopically.
2. Obstructive symptoms - Include straining, incomplete voiding, decreased urinary stream force and intermittent stream.
3. Irritative symptoms - Include urgency, dysuria, increased Frequency, nocturia, incontinence.
4. Pyelonephritis or hydronephrosis – when either 1 or both the ureters gets blocked completely or partially.
5. In advanced disease - signs and symptoms include flank, abdominal, bone or pelvic pain; cachexia or pallor; anorexia; renal failure; lower extremity edema; respiratory symptoms; suprapubic palpable mass.[21]

LOCATION OF UROTHELIAL TUMOURS:

Urothelial tumours can occur in any area of the bladder. Location in the decreasing order of involvement is lateral walls; posterior wall; trigone; neck; ureteric orifices; dome and anterior wall.[22]

SPREAD OF BLADDER CANCER

They are mostly single and localized to the bladder. However, the tumour may spread locally to the adjacent organs like ureters, prostate, uterus, vagina, intestines and rectum. Bladder cancer may metastasise via the lymphatics to the pelvic, paravesical, obturator or external iliac nodes and via the hematogenous route to the liver, lung, bone and adrenals.[23]

PATHOGENESIS OF UROTHELIAL NEOPLASMS:

WHO CLASSIFICATION OF UROTHELIAL TRACT TUMOURS - ANNEXURE-I

NONINVASIVE PAPILLARY UROTHELIAL NEOPLASIA

Over the years, several attempts were made to classify the urothelial carcinoma in order to grade the increasing degree of architectural disarray and the cytological changes seen in a single tumour type. A classification was proposed by the WHO and International Society of Urological Pathology (ISUP) in the year 1998. This was later recommended by the current 2016 WHO classification.[24] This classification divided neoplastic lesions into flat and papillary. The papillary neoplasms were further divided based on grade and invasiveness (based on involvement of lamina propria and Muscularis propria). The following types of papillary neoplasms were considered in the classification: [24]

1. Urothelial papilloma
2. Papillary urothelial neoplasm of low malignant potential (PUNLMP);
3. Low-grade papillary urothelial carcinoma
4. high-grade papillary urothelial carcinoma

The histological features of different papillary neoplasms are outlined in table 1.

Table 1. Histological features of different urothelial papillary lesions[25]

	PAPILLOMA	PAPILLARY NEOPLASM OF LOW MALIGNANT POTENTIAL	LOW-GRADE PAPILLARY CARCINOMA	HIGH-GRADE PAPILLARY CARCINOMA
ARCHITECTURE				
Papillae	Delicate	Delicate; sometimes fused	Fused, branching and Delicate	Fused, branching and delicate
Organization of cells	Identical to normal	Polarity identical to normal; any thickness; cohesive	Predominantly ordered, yet minimal crowding and minimal loss of polarity; any thickness; cohesive	Predominantly disordered with frequent loss of polarity; any thickness; often discohesive
CYTOLOGY				
Nuclear size	Identical to normal	May be uniformly enlarged	Enlarged with variation in size	Enlarged with variation in size
Nuclear shape	Identical to normal	Elongated, round–oval, uniform	Round–oval; slight variation in shape and contour	Moderate–marked pleomorphism
Nuclear chromatin	fine	Fine	Mild variation within and between cells	Moderate–marked variation both within and between cells with hyperchromasia
Nucleoli	absent	Absent to inconspicuous	Usually inconspicuous	Multiple prominent nucleoli may be present
Mitoses	absent	Rare, basal	Occasional, at any level	Usually frequent, at any level
Umbrella cells	Uniformly present	Present	Usually present	May be absent

The grading of bladder urothelial tumours has great prognostic significance. Papillomas have low recurrence rates and progression in carcinoma in situ (CIS) or invasive carcinoma while the high grade tumours have higher recurrence rates and progression. Urothelial papilloma of exophytic type consists of simple branching papillary architecture. They have histologically normal urothelial lining with neoplastic cells oriented upward or vertical to basement membrane and also have prominent umbrella cells.[26]

PUNLMP have a similar histologic appearance but differs from papillomas in having a thickened (hyperplastic) urothelial lining on low-power view in comparison with normal urothelium.[25, 27]

In the non-invasive papillary low-grade urothelial carcinoma, architecture and cytology appears orderly arranged. The cells are cohesive and evenly spaced and maintain polarity. Mild nuclear atypia having scattered hyperchromatic nuclei with mild variable size and shape of nucleus with less mitotic figures are seen in this type. They have a more complex and confluent papillae in comparison with papilloma and PUNLMP.

High-grade noninvasive papillary urothelial carcinoma show increased variation from normal architecture along with loss of polarity. They consist of dyscohesive cell having large hyperchromatic nuclei. Atypical mitotic figures are seen. Few highly anaplastic cells are sometimes seen. Compared to tumours that are low grade, they have a higher chance to invade the muscular layer, increased risk for progression and an increased metastatic potential.[27]

ENDOPHYTIC / INVERTED UROTHELIAL NEOPLASIA

Inverted urothelial papilloma is usually solitary, polypoidal and pedunculated with smooth borders. They present with hematuria and is commonly seen in adult and elderly males. The areas that get involved are the bladder neck, trigone of bladder or prostatic urethra.[28]

Microscopically, epithelial invagination is seen. Atypical features are not seen. Foamy cytoplasm may occasionally be present in epithelium.[29] They have scant connective tissue .Papillae maybe present focally or are absent.[30] Peripheral palisading of tumour cells arranged in trabecular pattern with central cellular spindling are noted in a few lesions while some other lesions showed islands of urothelial cells along with gland like and mucin secreting structures. [31] The inverted papilloma and carcinoma may co-exist together but they never predispose to carcinoma development.

All the non-invasive papillary lesions discussed above may have a similar non-invasive inverted growth with anastomosing cords and endophytic nests of urothelium along with their features. [32]

FLAT UROTHELIAL LESIONS WITH ATYPIA

According to the WHO/ISUP 2016 classification, [33] this category includes reactive urothelial atypia, urothelial atypia of unknown significance, urothelial dysplasia, and urothelial CIS.

Reactive urothelial atypia shows acute and chronic intraurothelial inflammation due to various causes like infection or calculi. The urothelium has basophilic appearance with monomorphic rounded and enlarged nuclei. They have fine nuclear chromatin with prominent nucleoli. Frequent mitosis may be seen. The inflammatory infiltrates may involve lamina propria. [32]

Urothelial CIS has disorganized urothelial cells which do not lie vertical to basement membrane. The cells have enlarged rounded nuclei having nuclear membrane which is irregular along with coarse nuclear chromatin and nucleoli may or may not be present. Atypical mitotic figures are seen.[34]

Lesions considered dysplasia usually have moderate cellular disorganization with only mild nuclear atypia and mild enlargement. Sometimes, mild cellular crowding or overlap is seen in normal urothelium but should not be considered as "atypical". To avoid confusion, some prefer to merge the categories of "atypia of unknown significance" and "urothelial dysplasia" into a single category "flat urothelial atypia, cannot exclude early flat neoplasia (dysplasia)." Presence of increased nuclear enlargement, significant nuclear hyperchromasia along with brisk mitotic activity generally strongly suggests CIS and distinguishes atypia/dysplasia from CIS. [23]

INVASIVE UROTHELIAL CARCINOMA

MACROSCOPY

Invasive urothelial carcinoma can be unifocal or sometimes multifocal. They may be large, polypoid or sessile and even ulceroinfiltrative.

MICROSCOPY

Invasive urothelial carcinoma typically shows irregularly distributed nests of urothelial cells having either rounded or sharped contours. Stromal response includes fibrosis, inflammatory reaction, desmoplasia, myxoid changes or stromal retraction clefts. The tumour may show early invasion involving lamina propria. They consist of urothelial cells arranged in small solid nests or clusters having cytoplasmic eosinophilia

with surrounding retraction. When the muscularis propria by infiltrated by the tumour, it is diagnostic of malignancy. [35]

Invasive urothelial carcinomas sometimes show squamous or sometimes glandular differentiation. Many variants/types of urothelial carcinoma are known. Some variants with important clinical significance include:

MICROPAPILLARY CARCINOMA OF BLADDER

Histologically, this variant of invasive urothelial carcinoma mimics ovarian serous carcinoma. It consists of "back-to-back" retraction spaces having multiple epithelial nests in a single retraction space. Micropapillary carcinoma variant usually invades into muscularis propria. It can show lymph node metastases.[36]

PLASMACYTOID CARCINOMA OF BLADDER

Plasmacytoid variant is characterized by discohesive neoplastic cells which are monomorphic and round growing in clusters or singly. These neoplastic cells have homogeneous eosinophilic cytoplasm commonly with intracytoplasmic vacuoles and a nucleus that is an eccentrically placed. Cytokeratins, CD138, CK20 and GATA3 are expressed by plasmacytoid variant of bladder carcinoma but are less sensitive to p63.[37] Plasmacytoid carcinoma may involve serosal surfaces and ovaries. They may recur as malignant effusions. The adipose tissue may be involved.[38]

NESTED UROTHELIAL CARCINOMA

Nested carcinoma consists of rounded nests of urothelium which have cytologically bland appearence. The irregular distribution of these nests in the suburothelial tissues distinguishes it from benign von Brunn nests. Central cystic changes are seen sometimes.[39]

LYMPHOEPITHELIOMA-LIKE CARCINOMA

This variant looks like a nonkeratinizing carcinoma having a heavy inflammatory infiltrate especially in pure lymphoepithelioma-like carcinoma. Most cases show focal or extensive change in a tumour having otherwise the appearance of a urothelial carcinoma.[40]

SARCOMATOID / SPINDLE CELL / METAPLASTIC CARCINOMA

This type of bladder cancer has both malignant epithelial component as well as spindled "sarcoma-like" appearing areas in the carcinoma. Features of rhabdomyosarcoma, chondrosarcoma, osteosarcoma or liposarcoma may also be exhibited. Metastases can occur to lymph nodes nearby.[41]

IMMUNOHISTOCHEMICAL FEATURES

The urothelial carcinoma has got a heterogeneous immunophenotype, especially across variant forms. The neoplastic cells are positive to markers like p63, CK20, CK7 and HMWCK.[42] GATA3 marker also shows nuclear staining in urothelial carcinoma.[43] Uroplakin II is another marker showing positive staining for urothelial carcinoma.[44, 45]

TREATMENT:

The management of bladder carcinoma is based on the following factors: [46]

- The extent, stage and microscopic grade of tumour.
- Patient's age and surgical risk.
- The presence of CIS with bladder.

The common treatment is total cystectomy for extensive CIS. The recommended treatment for small localised lesions is intravesical chemotherapy which may cause temporary or complete remission.[47]

Intravesical immunotherapy with the BCG can be done for "superficial" bladder carcinomas (pTa or pT1 papillary and/or CIS). This therapy has bought reduction in the rate of recurrrence in some cases.[48]

Urothelial carcinomas having no muscularis propria invasion are treated initially with Transurethral resection of bladder tumour (TURBT) and are supplemented with intravesical chemotherapy in high-grade or invasive pT1 tumours having multiple or recurrent tumours.[49, 50]

Irrespective of grade, Urothelial Carcinomas with muscularis propria invasion and tumours resistant to conservative therapy undergo radical cystectomy, with or without preoperative chemotherapy.[47]

Radical cystectomy consists of removing bladder along with prostate as well as seminal vesicles and also perivesical tissues around it in males. In females, it consists of removal of bladder and uterus along with the fallopian tubes as well as ovaries and also the anterior vagina and urethra.[51] Sometimes, radical cystectomy may be combined with removal of pelvic lymph nodes.[52]

In the patients with advanced bladder carcinoma, chemotherapy is given along with radical cystectomy has increased the survival of the cancer patient.[53]

PROGNOSIS:

Various prognostic factors for bladder cancer include: [20]

1. Depth of tumour and stage

This is an important factor for prognosis. With increase in depth of invasion, the prognosis becomes less favourable. In advanced stages, the lymph nodes or other structures are involved and hence have a lesser prognosis compared to the early stages.[54]

2. Grade

Higher grade bladder cancers have an increased chance to spread and hence have poor prognosis compared to bladder cancers of lower grade. [20, 54]

3. CIS

Less favourable prognosis is seen in CIS cases and these have greater risk to develop into invasive bladder cancer. [20]

4. Type of tumour

Papillary urothelial carcinomas of bladder are known to have best prognosis. Small cell carcinoma, adenocarcinoma as well as squamous cell carcinoma of bladder have poor prognosis. [20]

5. Number of tumours

Increased number of tumour areas in the bladder, the less is the prognosis.

6. Size of tumour

Large size of tumour has a poor prognosis while smaller size of tumour has a better prognosis. [55]

7. Recurrence

The more the recurrence of a tumour, poor is its prognosis.[20]

8. Lymphovascular invasion (LVI)

Prognosis is poor if there is LVI seen as such cancers have increased risk of spreading to other locations in the body. [54]

TNM CLASSIFICATION OF BLADDER CANCER- ANNEXURE-II

PROSTATE

EMBRYOLOGY OF PROSTATE:

The prostate gland is formed from the buds on the prostatic urethral epithelium. The secretory epithelium of prostate gland arises from these buds. The inner glandular zone is formed from buds in the mesoderm of prostatic urethra while outer glandular zone is formed from buds in the endoderm of prostatic urethra.[12] The outer zone differentiates first followed by the inner zone. The inner zone gets involved during senile hypertrophy of prostate while outer zone gets involves in carcinoma. The surrounding mesenchyme forms the capsule, muscles and connective tissue of gland.[12]

ANATOMY OF PROSTATE GLAND:

The prostate is an accessory gland which is small sized and forms part of reproductive system in males. It is positioned in the cavity of pelvis surrounding the urethra immediately below bladder neck. The rectum is situated posterior to the organ and anterior to it lies the pubic symphysis. It has firm consistency because of presence of dense stroma which is fibromuscular.[14]

It appears like an inverted cone in shape which measures about 3cm in length, 4cm in width at the base and 2 cm in thickness. It has a weight of about 8 g. The prostatic gland secretion constitutes a major part of seminal fluid. The prostatic secretion makes semen thinner and also contains spermine, a hormone – like substance that helps in the motility of sperms. The secretion from the prostate also helps the sperm cells to function properly and maintains fertility in males. The secretion has alkaline nature and helps in neutralizing the vaginal secretions which are acidic. The paraurethral glands of Skene replace the prostate in females.[14]

The prostatic urethra moves through the gland vertically in the junction between anterior 1/3rd and posterior 2/3rd of the gland. The ejaculatory ducts move downwards and forwards with the gland and terminates into the prostatic urethra.[16]

The prostate gland earlier had 5 lobes which were anterior, posterior, median and 2 lateral lobes. But now, it has been described to have 3 lobes only: - 1 median and 2 lateral lobes.[15]

GROSS FEATURES OF PROSTATE:

The prostate gland has an apex pointing downwards with a base and four surfaces which include 2 inferolateral surfaces, an anterior surface and a posterior surface.[16]

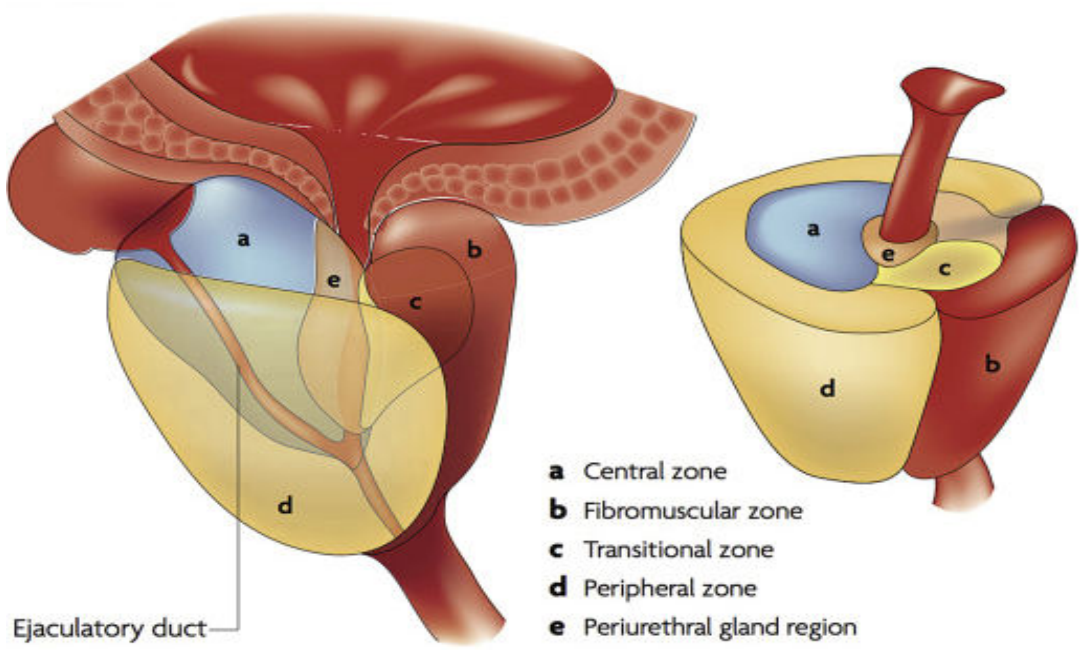
a) Apex: The downward directed apex lies at the area between prostatic and membranous parts of urethra.
b) Base: The base is continuous with bladder neck and is directed downwards
c) Surfaces:
    - **Anterior** surface contains fibrous tissue and is situated about 2 cm behind the symphysis pubis. The retropubic fat separates the surface from pubic symphysis. The puboprostatic ligaments join superior part of anterior surface with bones of pubis. The urethra pierces at inferior part of anterior surface.
    - **Posterior** surface of prostate is a triangular area lying about 4 cm from the anus. Fascia of Denonvilliers separates the surface from the rectum. The ejaculatory duct moves through this surface on each side at its upper border. This surface is felt easily by palpation in digital rectal examination
    - I**nferolateral** surfaces lie in relation to side walls of pelvis.

ZONES OF PROSTATE:

According to McNeal, the gland is divided into the following zones: [14]

1) PERIPHERAL ZONE: It lies posteriorly and constitutes 70% of prostatic glandular tissue. This zone is prone to get involved in prostatic cancer.
2) CENTRAL ZONE: This zone arises from the wolfian duct. It lies posterior to urethral lumen and ejaculatory ducts is below it. It forms 25% of prostate tissue and not involved in disease.
3) TRANSITION ZONE: It is periurethral in location constituting only 5 % of prostatic glandular tissue and gets involved in benign prostatic hyperplasia.

Figure 4: Zones of prostate [56]



(**Image taken from:** *De Marzo AM, Platz EA, Sutcliffe S, Xu J, Grönberg H, Drake CG, et al. Inflammation in prostate carcinogenesis. Nat Rev Cancer. 2007; 7(4):256-69*)

The "central" gland is formed by combination of central and transition zone.

CAPSULES OF PROSTATE:

The prostate has 2 capsules: [16]

- **True capsule:** A fibromuscular structure formed from the condensed peripheral part of gland. It continues with the glandular stroma.
- **False capsule:** Formed from endopelvic fascia. Anteriorly, the capsule continues as puboprostatic ligaments and posteriorly, rectovesical fascia of Denonvilliers gives rise to this capsule and is avascular.

The prostatic plexus lies in between the false and true capsules.

LIGAMENTS OF PROSTATE:

The ligaments of prostate provide support to the gland and are situated between the fibrous capsule and back of pubic bone. They include: [14]

1. Medial puboprostatic ligaments – close to apex
2. Lateral puboprostatic ligaments – close to base

ARTERIAL SUPPLY OF PROSTATE:

A large subcapsular plexus and a small periurethral plexus derived from the middle rectal, inferior vesical and internal pudendal arteries supply gland.[15]

VENOUS DRAINAGE OF PROSTATE:

Veins supplying prostate form a plexus which communicates with the vesical plexus and internal pudendal vein. The prostate carcinoma can involve skull and vertebral column due to absence of valve in the communication between prostatic and vertebral venous plexus.[14]

LYMPHATIC DRAINAGE OF PROSTATE:

1) Internal iliac and sacral nodes
2) External iliac nodes.[15]

NERVE SUPPLY OF PROSTATE:

The prostate has both sympathetic and parasympathetic nerves. On stimulation, the prostatic secretions are produced and discharged. The inferior hypogastric plexus gives rise to the prostatic plexus of nerves.[16]

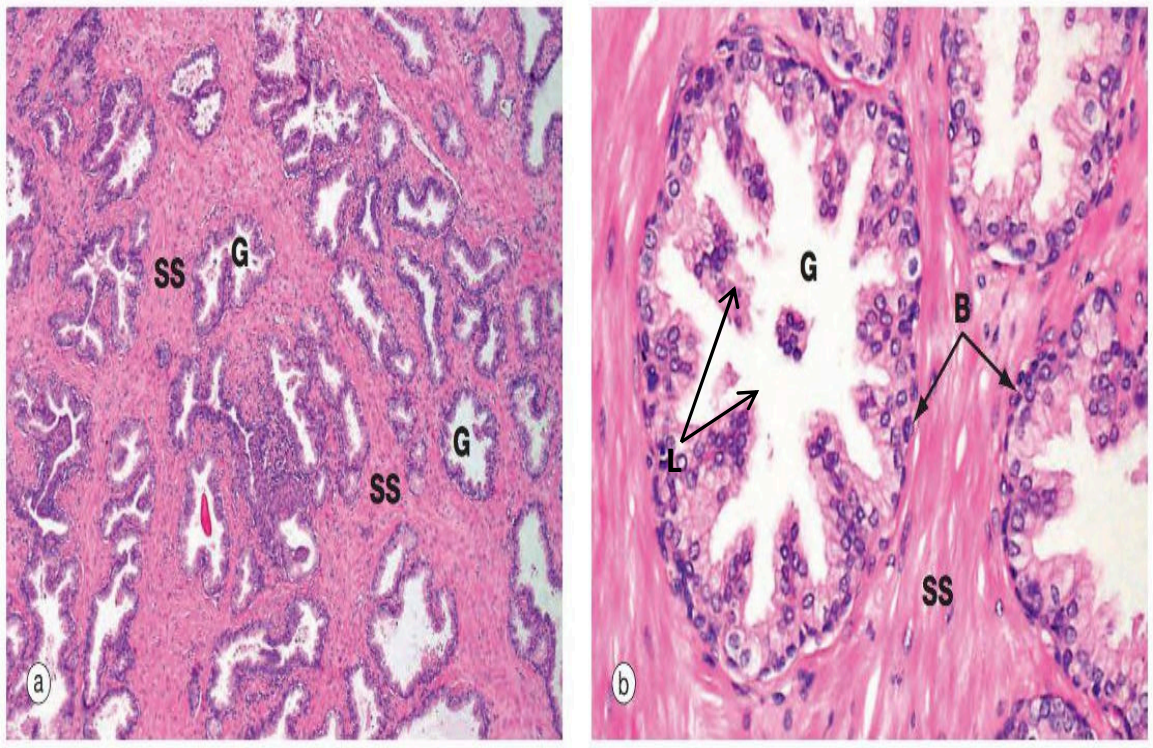
HISTOLOGY OF PROSTATE:

Microscopically, a normal prostate consists of gland and stroma. The glandular system forms complex architectural pattern. The glands are of variable size and shape having convoluted pattern with epithelium thrown in to papillary folds. The epithelium of the prostate glands consists of luminal cells which are tall columnar cells having secretory function with prominent basal nuclei and pale staining cytoplasm. Small flat cigar shaped basal cells are seen below the luminal secretory epithelium lying along basement membrane with the long axis in the direction of basement membrane.[18]

The stroma is dense and fibromuscular and consists of smooth muscle bundles and connective tissue fibres mixed together within the stroma.[17]

Some the glands contain proteinaceous prostatic secretions. In some glands, these secretions accumulate to form corpora amylacea which are spherical concretions. As the age increases, they become more in number and later become calcified.[18]

Figure 5. Histology of normal prostate gland [18]



SS – Supporting stroma, G- Glands, B- Basal cells, L- Luminal cells

(**Image taken from:** *Young B, O'Dowd G, Woodford P. Wheater's Functional Histology, 6e. New Delhi, India: Elsevier; 2014*)

PROSTATE ADENOCARCINOMA

Prostate cancer ranks 2nd among the most frequently diagnosed cancer in men worldwide as per the GLOBOCAN 2018.[1] In India, prostate cancer ranks 16th in terms of incidence in the year 2018.[2] There is an increase in trend observed for five year prevalence rates in India for prostate cancers between the years 2018 and 2020. It has risen from 6.78% to 9.47% respectively.[2,3]

Prostate adenocarcinoma is seen commonly in men more than 50-60 years of age. The black population is more susceptible to prostate adenocarcinoma than the whites. Prostate cancer is uncommon in Asians.[20]

ETIOLOGY AND PATHOGENESIS:

1. Age: The incidence of this cancer ranges from 20% in men in their 50s to about 70 % in men in the age group of 70 - 80yrs.
2. Environmental factors: Prostate cancer risk is increased in those who consume more fat or carcinogens found in charred red meats while dietary products like soy products, antioxidants, vitamin D and lycopenes that are found in tomatoes may reduce the risk. [20]
3. Family history: There is increased risk of 2-3 fold to develop prostate cancer in men with a 1st degree relative suffering from prostate cancer.
4. Racial factors: There is increased incidence of developing the cancer in African Americans than in Americans and Asians.[57]
5. Hormonal factors: Androgens promote growth and survival of prostate cancer cells.[57] These bind to androgen receptors and induce expression of pro-growth and pro-survival genes. Anti-androgens will induce regression of the disease.

Increased PI3K/AKT signalling activation is seen in patients who have become resistant to antiandrogen therapy. Increased of risk of developing the cancer is seen in men with germline mutations of the tumour suppressor gene BRCA2 and HOXB13 that encodes for transcription factors which regulate the development of prostate in men. Overexpression of ETS transcription factors may cause the upregulation of matrix metalloproteases and result in the normal epithelial cells of prostate gland to become more invasive. Amplification of the 8q24 locus having the MYC oncogene and deletion involving PTEN tumour suppressor gene are seen in prostate cancer. In the later stages of prostate cancer, loss of TP53 by deletion or mutation and RB gene deletions are commonly seen.[20, 23]

Epigenetic alterations commonly seen in prostate cancer is the hypermethylation of GSTP1 or glutathione S-transferase gene down-regulating the GSTP1 expression. Tumour suppressor genes like genes involved in regulation of cell cycle (CDKN2A, RB), Wnt signalling pathway suppression (APC) and maintenance of genomic stability (MSH2, MLH1) are also silenced by epigenetic alterations in prostate cancers. [20]

SPREAD OF PROSTATE ADENOCARCINOMA:

DIRECT SPREAD:

Direct spread occurs into the capsule of prostate and extends to the periprostatic tissue, seminal vesicles, neck of bladder, trigone and ureteral openings.[20]

METASTATIC SPREAD:

- Hematogenous spread:

The cancer spreads to lumbar spine and pelvic bones via the prostatic venous plexus. The bony metastasis is usually osteoblastic. Metastasis to brain, kidney and lung may also occur via hematogenous route.

- Lymphatic spread:

By the lymphatic route, metastasis occurs to the obturator lymph nodes and to para-aortic, sacral and iliac lymph nodes.[20]

CLINICAL FEATURES OF PROSTATE ADENOCARCINOMA:

In early stages of cancer, majority of the cases are asymptomatic and are usually discovered by rectal examination or elevated serum PSA level.

Presence of symptoms is seen in advanced and metastatic stages of the cancer. The clinical manifestations include: [20, 58]

1. Locally advanced disease: Results in lower urinary tract symptoms like:

- Increased frequency of urination
- Weak flow of urine
- Difficulty in initiating urination
- Urinary retention
- Dribbling of urine
- Hematuria

2. Advanced disease (spread to the regional pelvic lymph nodes):

- Edema of lower extremities
- Pelvic/ perineal discomfort

3. Metastatic disease:

- Bone pain
- Symptoms due to compression of spinal cord
- Paraperesis
- Hematuria due to prostatic urethra or trigone involvement
- Renal impairment as result of prolonged bladder outlet obstruction

PATHOGENESIS OF PROSTATE ADENOCARCINOMA:

WHO CLASSIFICATION OF PROSTATE TUMOURS - ANNEXURE-III

Histologically, there are two major categories of prostatic carcinomas that have been described: [23]

1. Acinar
2. Ductal

ADENOCARCINOMA OF ACINAR TYPE

Prostatic adenocarcinomas usually arise in peripheral zone in the posterior location which can be palpated on rectal examination and may later involve anterior regions of the prostate. Prostatic transition zone or central zone involvement is rare.[59]

Grossly, sometimes the tumour may not be evident but usually seen as a grey-yellow, poorly defined, firm lesion. Cut surface of prostate shows a neoplastic area which will be firm and gritty. The lesion is easily felt than seen.[23]

A variety of morphological patterns are described. These include: [60]

1. Medium-sized glands
2. Small glands
3. Diffuse individual cell infiltration
4. Cribriform
5. Poorly formed glands (pattern of small tumour nests)
6. Glomeruloid pattern

Carcinomas that contain medium-sized glands may be described as having closely spaced glands with smooth inner luminal surface. The luminal cells have prominent cytoplasm with nuclei oriented against the basement membrane. This pattern has scanty intervening stroma. Carcinomas consisting of small glands have small sized

glands with regular round configuration. They are seen as expansive nodules or as an infiltrative individual glands present in between benign glands. [61, 62]

Both of these patterns also show enlargement of the nucleus with hyperchromasia and prominent nucleoli or macronucleoli (measuring >1 μ in diameter). Mitoses are sometimes seen.[63]

The pattern of diffuse cell infiltration is usually rare. The cribriform pattern has small - medium sized glands having intraluminal proliferations which are complex. Glomeruloid pattern contains ball-like tumour cell clusters within lumen and is considered characteristic of malignancy.[64]

All these patterns are seen in combinations. Sometimes, high grade prostatic carcinoma may show squamous metaplasia.

Variations of prostatic adenocarcinoma include:

1. Foamy gland carcinoma:

The tumour cells of this variant are cuboidal to columnar. They have fine granular cytoplasm and small or pyknotic nuclei with inconspicuous nucleoli. Sometimes, there is prominence of clear or foamy (xanthomatous) cytoplasm due to massive accumulation of lipids and hence, the name. They usually have low Gleason score.[65]

2. Pseudohyperplastic variant:

The tumour has a microcystic appearance on low power. They contain hyperplastic glands having branching and papillary infoldings. In addition, features like enlargement of nucleus, nuclear orientation at the basement membrane, macronucleoli and intraluminal crystalloids are also seen in this variant. Mitoses are also seen.[66]

3. Adenocarcinoma having atrophic features:

They have infiltrative growth pattern and contains tumour cells having reduced cytoplasm and nuclei occupying the entire cell. Nuclear enlargement and macronucleoli are also seen.[67]

4. PIN-like adenocarcinoma:

Some variants may mimic high grade prostatic intraepithelial neoplasia (PIN) by having medium to large glands with a stratified epithelial lining.[68] However, these carcinomas have a crowded arrangement of glands unlike in PIN. Neoplastic cells with pseudostratified columnar are also seen.

5. Aberrant p63 expressing carcinoma:

This is a rare form of prostatic adenocarcinomas showing atrophy with scant cytoplasm and slit-like luminal spaces having eosinophilic secretions in the lumen occasionally. The neoplastic secretory cells in the variant express nuclear p63.[69]

DUCTAL ADENOCARCINOMA

Ductal adenocarcinoma is another major type of prostatic carcinoma usually seen at periurethral location. Grossly, they appear as a polypoid villous lesion with an infiltrative urethral component. Microscopically, they show papillary and cribriform architecture lined by columnar pseudostratified epithelium. The basal cells may remain intact. [70]

Those prostatic carcinomas that present as macrocystic tumours may show ductal features. The ductal adenocarcinomas are usually at more advanced stage at

presentation and have an increased short-term survival rate when compared to the typical acinar carcinomas. [71]

OTHER MICROSCOPIC TYPES:

1. Prostate adenocarcinoma with neuroendocrine differentiation:

This subtype of prostatic adenocarcinoma is rare and has Paneth cell–like neuroendocrine differentiation. They may grow in nests, cords and as single cells. This carcinoma contains patchy or diffuse presence of cells having eosinophilic cytoplasmic granules.[72] Small cell neuroendocrine carcinoma is very aggressive and has a large number of apoptotic cells. Long term hormonal therapy for prostate adenocarcinoma can give rise to large cell neuroendocrine carcinoma

2. Mucinous (mucin-secreting) adenocarcinoma:

This tumour contains large amounts of intracellular and extracellular mucin. This comprises about 25% or more of the tumour. Various patterns of the tumour is seen which include microglandular, solid, cribriform, comedo and hypernephroid patterns.[73] This tumour stains positive for PSAP and PSA. Bone metastases are rarely seen. It does not depend on hormone and show poor response to radiation therapy. Occasionally, Paneth-like neuroendocrine cells may be seen.

3. Signet ring carcinoma:

This is a highly malignant neoplasm with tumour cells arranged in acinar, solid or single file pattern. These tumour cells show signet ring configuration due to intracellular accumulation of mucin.[74] Ultrastructurally, the cells contain microvilli which line the intracytoplasmic lumina.

4. Adenosquamous carcinoma:

They are seen de novo or after radiation or hormonal therapy of usual adenocarcinomas.[75]

5. Squamous cell carcinoma (SCC):

This type may occur de novo or after hormonal therapy. This type of carcinoma is usually seen as a well-circumscribed nodule in the prostatic transition zone. Pure SCC of the prostate is rare and are usually seen closely related to adenosquamous carcinoma.[76]

6. Adenoid basal cell tumour:

Adenoid basal cell tumour resembles the adenoid cystic or basal cell carcinoma of the salivary gland. Microscopically, it has an expansile growth pattern with multinodularity and cribriform architecture. The surrounding stroma is usually fibromyxoid. Foci of basal cell hyperplasia and squamous differentiation are common findings.[77]

7. Basal cell carcinoma:

This type is highly aggressive and MYB rearrangement has been seen in some cases.[77]

8. Lymphoepithelioma-like carcinoma:

This type of tumour has an appearance similar to nasopharyngeal lymphoepithelioma.[78]

9. Sarcomatoid carcinoma:

Sarcomatoid carcinoma variant has a combination of carcinoma with sarcomatoid elements which includes spindle cell or giant cell features and sometimes cartilage, bone or skeletal muscle differentiation.[79]

STAGING AND GRADING:

The initial spread of prostate adenocarcinoma occurs within the different compartments of prostate. It may involve the duct, acini, blood vessels, fibromuscular stroma and perineural spaces.[80] Extraprostatic extensions are common and may involve the bladder, apex of the gland, prostatic urethra and seminal vesicles.[81] Specimen of prostate adenocarcinoma received as a whole is staged depending on the histologic evaluation for extraprostatic extension. The carcinoma infiltrates beyond the soft tissue at interface between the prostate and adipose tissue. The grading of the prostate has been discussed in the table below.

The preferred microscopic grading system that is currently in use was introduced by Dr. Donald Gleason. This grading system is developed taking in to account the following factors evaluated on low-power microscopic examination: [82, 83]

1. The amount of glandular architectural differentiation
2. The growth pattern of tumour in relation to stroma

The primary tumour pattern that is predominant is graded from 1-5 and secondary pattern if present, is also graded from 1-5. The two values are then added to get Gleason score. However, if there is same pattern (or primary pattern) throughout the tumour, then the number is multiplied by 2 to get Gleason score.[84] Tertiary (or minor) pattern is reported only in cases where it is of a higher grade.[85]

In modern practice, the Gleason patterns 1 to 2 are not clinically significant and hence, the grading system begins at the Gleason score 3 + 3 = 6. According to 2014 criteria, Gleason pattern 3 constitutes only the glands which are well formed and have central lumina.[86] The Gleason pattern 4 consists of a heterogeneous pattern of cribriform glands, glomerulations, fused glands and glands that are poorly

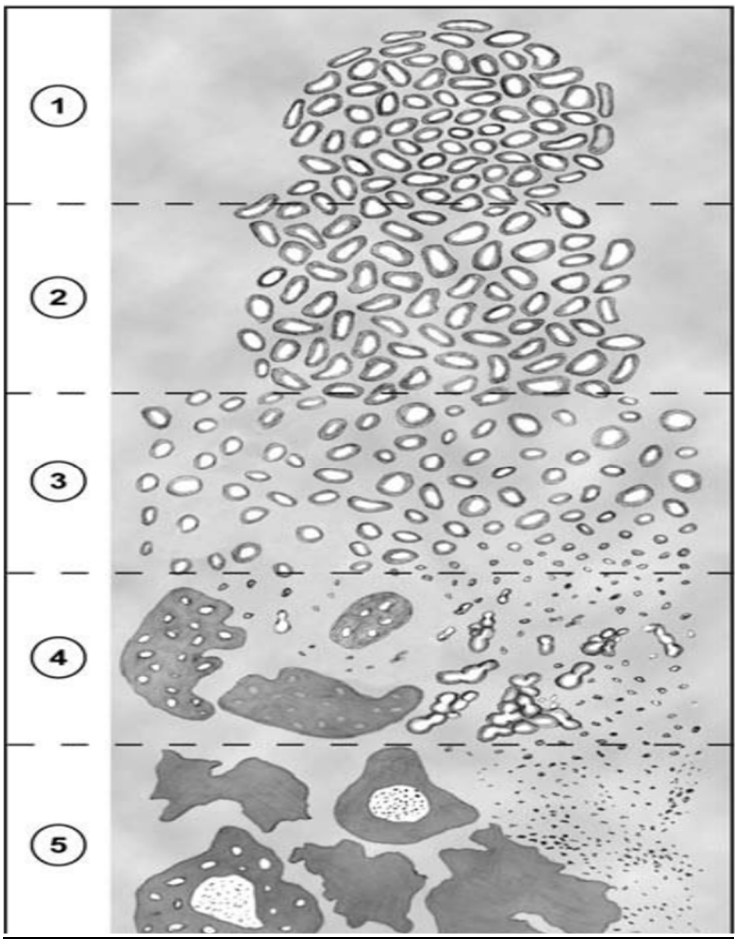
formed. Gland formation is absent in Gleason pattern 5 and they are characterized by sheets, cribriform glands having central comedonecrosis and single infiltrating cells which mimics a lobular breast cancer.

Grade Group system has recently been developed as a better system to report the Gleason score categories as shown the table below.[86] It is observed from various studies that grading of prostatic adenocarcinoma has correlated well with survival rate, lymph node and bone metastases, PSAP (prostate specific alkaline phosphatase) and PSA (prostate specific antigen) levels, clinical and pathologic staging and response to therapy.[87]

Table 2. Gleason grade group and Gleason scoring with histological features [86]

GRADE GROUP	GLEASON SCORE	HISTOLOGICAL FEATURES
1	≤3 + 3 = 6	Only individual well-formed and discrete glands
2	3 + 4 = 7	Predominantly well-formed glands with lesser component of poorly formed glands, fused glands, glomerulations or cribriform glands
3	4 + 3 = 7	Predominantly poorly formed glands, fused glands, glomerulations or cribriform glands with lesser component of well-formed glands (>5%)
4	4 + 4 = 8	Only poorly formed glands, fused glands, glomerulations or cribriform glands
	3 + 5 = 8	Predominantly well-formed glands with lesser component of sheets, cribriform glands with comedonecrosis or single cells
	5 + 3 = 8	Predominantly sheets, cribriform glands with comedonecrosis or single cells with lesser component of well-formed glands (>5%)
5	≥4 + 5 = 9	Only sheets, single cells or cribriform glands with comedonecrosis

Figure 6: Schematic representation of Gleason grading system [88]



(**Image taken from:** *Epstein JI, Allsbrook WC, Jr., Amin MB, Egevad LL. Update on the Gleason grading system for prostate cancer: results of an international consensus conference of urologic pathologists. Adv Anat Pathol 2006; 13:57–9*)

IMMUNOHISTOCHEMISTRY:

Immunohistochemical markers staining the prostatic epithelium like PSAP and PSA are demonstrable in routinely processed tissues with polyclonal or monoclonal antibody.[89, 90] These markers help to identify whether the metastatic tumours are of prostatic origin.

NKX3.1is another prostatic epithelial marker having better sensitivity and specificity than PSAP and PSA and shows nuclear positivity.[91]

PSAP, PSA, NKX3.1 along with HMWCK, p63 or GATA3 can be used to differentiate poorly differentiated cases of prostate and urothelial carcinomas. [92]

PSMA (prostate specific membrane antigen) is a marker expressed in prostate adenocarcinomas. A cytoplasmic protein P504S, isolated through microarray screening is a marker for prostatic carcinoma with high sensitivity and shows strong circumferential luminal staining.[93]

High-molecular-weight keratins are present in the basal cells in the prostatic glands and are identified by Antibody 34βE12. It helps to differentiate the benign or atypical glands from prostate adenocarcinomas. Markers like keratin 5/6 and p63 can also be used for this purpose. [94]

The malignant glands of the prostate adenocarcinoma show P504S reactivity in the luminal cells but the two basal cell markers are negative, whereas benign glands will not stain for P504S and show reactivity to the two basal cell markers. [95]

TREATMENT:

The treatment for a localized prostatic carcinoma includes radical prostatectomy, external radiation therapy, brachytherapy, cryotherapy or high-intensity focused ultrasound (HIFU) and active surveillance.[96]

Hormonal therapy includes the oestrogens, luteinizing hormone–releasing hormone (LH-RH) analogues and antiandrogens. Hormonal therapy has replaced orchiectomy as a palliative measure in the case of locally advanced as well as metastatic tumour.[97] Systemic chemotherapy which can be used in prostate adenocarcinoma includes chemotherapeutic agents like docetaxel and cabazitaxel in addition to next-generation hormonal therapies like abiraterone and enzalutamide.[98, 99]

PROGNOSIS:

Various parameters evaluated to predict prognosis in the prostate adenocarcinoma patients include: [23]

1. Clinical stage

2. Pathologic stage:

An important indicator of extent of tumour. It helps in the prediction of prognosis accurately. There is strong correlation between levels of tumour invasion into the capsule of prostatic and grade, volume and recurrence rate of the tumour. In cases with nodal metastases, there is worse prognosis and also when tumours are multiple, when they are detectable grossly, when they have overall large volume and when they extend beyond the capsule.[100]

3. Microscopic grading:

The clinical or pathologic staging directly co-relates with microscopic grading system. Gleason grading system is a significant predictor of prognosis for prostatic carcinoma.[101]

4. Surgical margins:

Involvement of surgical margin is an indicator of increased risk of tumour progression.[102]

5. Tumour volume:

Commonly used methods to estimate tumour volume include visual estimation of percentage of gland involvement or providing the greatest linear dimensions of tumour. In core needle biopsies, even rough estimations of tumour amount like percentage of

cancer or number of cores showing positive tumour cells have a prognostic significance.[103]

6. PSA serum levels:

The serum level of PSA is an indirect indicator of tumour volume and response to therapy and hence related to the prognosis of prostate adenocarcinoma.[104]

7. Race:

Mortality due to prostate adenocarcinoma is seen more commonly in African men than in the white males.[105]

8. Lymphovascular invasion:

The tumour permeates vascular channels and this has important correlation with Gleason score, involvement of seminal vesicle and probability of tumour progression. Also, involvement of lymph vessels in peritumoural area has increased chance of metastases to lymph node in the region.[106]

9. Neuroendocrine features:

Prostatic cancer with neuroendocrine features is associated with poor differentiation and poor prognosis of the cancer.[107]

10 . Prominent reactive stroma:

Recurrence is usual in prostate cancers with abundant reactive stroma.[108]

11. Androgen-receptor status:

Aggressive clinicopathologic features have been associated with increased levels of androgen receptor.[109]

12. Proliferation index:

The Ki-67 proliferation index of prostatic carcinoma may predict mortality associated with tumour in both limited disease and in tumours with lymph node metastases.[110]

13. PTEN loss:

In advanced stage of the disease, PTEN inactivation is common and hence, is a suggestive marker of aggressive prostate cancer.[111]

TNM CLASSIFICATION OF PROSTATE ADENOCARCINOMA - ANNEXURE-IV

GATA3 IMMUNOHISTOCHEMICAL MARKER:

GATA3 belongs to family of transcription factor that binds to DNA sequence (A/T) GATA (A/G) present in the genes promoters and are involved in the activation and the repression of target genes. This transcription factor has zinc finger binding domain that has an essential role in promoting the proliferation, development as well as differentiation of cells in many tissues including the cell types of glandular luminal epithelial cells in mammary gland, T lymphocytes, thymocytes, hair follicles of skin, adipose tissue, kidney and sympathetic nervous system. [10, 11]

The GATA3 marker is associated with cancers like the breast and colorectal cancers.[11, 112] It has now been recognized as an immunohistochemical (IHC) marker for urothelial carcinomas which can help to differentiate it from prostatic adenocarcinomas. Markers like CK7 and CK20 can be used to differentiate both the carcinomas as the prostate adenocarcinoma stains only rarely to CK7 and CK20 unlike urothelial carcinomas .[113] CK7 /CK20 phenotype of prostate adenocarcinoma is found to

be heterogenous in high grade carcinoma cases in various studies limiting the utility of these markers to differentiate the two entities.[114]

In a prospective study done by Agarwal H et al at KGMU, Lucknow, 74 urothelial carcinoma cases, 10 prostatic adenocarcinoma cases and 10 renal cell carcinoma (RCC) cases were stained with GATA3 immunostain. GATA3 expression was observed in 77% of urothelial carcinoma cases, whereas RCC and prostatic adenocarcinomas were negative for GATA3. Sensitivity and specificity were 78.7% and 100% respectively for urothelial carcinoma.[6]

In another study conducted at Benha University, Egypt, immunohistochemistry for GATA3 and p63 was performed on 30 cases each of high-grade urothelial carcinoma and high-grade prostatic adenocarcinoma. GATA3 and p63 were positive in 25 (83.3%) and in 27 (90%) of high-grade urothelial carcinoma, respectively. Of the 5 cases negative for GATA3, 3/5 was positive for p63 while 2/5 was negative for both markers. Prostatic adenocarcinomas did not express either GATA3 or p63.[4]

In a retrospective study done by Abdullah WH et al at Babylon training canter for pathology, Iraq, 51 cases of urothelial carcinoma and 15 cases of prostatic adenocarcinoma were stained with GATA3 immunohistochemical stain. 96% cases of urothelial carcinoma were GATA3 positive. No correlation between expression of GATA3 and clinicopathological parameters was observed including grade and stage. No GATA3 positivity observed in prostate adenocarcinoma.[5]

In the study done by Mohammed KH et al, it was found that GATA3 positivity was 70.8% (56/79) for invasive urothelial carcinomas. All RCC and prostate adenocarcinomas did not stain for the marker. The GATA3 staining was considered

positive when there was brown nuclear staining in the malignant cells. Increased GATA3 expression was found correlating with larger tumour size of urothelial carcinomas. No correlation between expression of GATA3 with other clinicopathological parameters observed in urothelial carcinomas.[9]

MATERIALS AND METHODS

Study design: Cross sectional study

Study population and data collection: All the specimens of urothelial carcinoma and prostatic adenocarcinoma received at the surgical pathology laboratory at KLE'S DR. PRABHAKAR KORE HOSPITAL and MEDICAL RESEARCH CENTER, BELAGAVI between January 2018 and December 2020.

For retrospective cases (January 2018 to December 2019), archival data as well as tissue blocks were retrieved from the storage of the pathology laboratory.

Sample size: Total 80 cases – 40 cases each for urothelial carcinoma and prostate adenocarcinoma.

Selection criteria:

Inclusion criteria:

All specimens which were diagnosed histopathologically as urothelial carcinoma and prostate adenocarcinoma.

Exclusion criteria:

1. All inadequate and improperly preserved specimens.
2. Cases reported as premalignant lesions.

Ethical clearance

The ethical clearance was acquired from Institutional Ethics Committee, JNMC, Belagavi prior to the commencement of study.

Method of data collection

Procedure: All the biopsies and whole specimens of urothelial carcinoma and prostate adenocarcinoma received at the histopathology laboratory were collected, numbered and kept for fixation in 10 % formalin overnight. The specimens were taken the next day for grossing and representative sections of tissue were given in different capsules. These capsules were taken for processing in the tissue processor. The tissues in the capsules underwent the process of dehydration in upgraded alcohol solutions, clearing in xylene and impregnation with paraffin wax in the tissue processor.

The tissues were then taken from the capsule and embedded in molten wax for block preparation. Sections measuring 3-4 microns each were cut using microtome and taken on to the slides. One of the slides was stained using H & E stain and air dried for histopathological evaluation. The slides for IHC were pre-coated using Poly-L-Lysine and stained for IHC using specific mouse monoclonal antibody to GATA3. For positive control, human neuroblastoma tissue was taken and for negative control, IHC staining was done without the use of primary antibody. After dipping slides in xylene, they were mounted with a coverslip using DPX.

For retrospective cases, data as well as tissue blocks were retrieved from the storage. The tissue blocks were cut and slides were prepared for staining. Both H & E and GATA3 IHC staining procedures were performed as above and mounted.

All the slides were examined and reported using the WHO/ISUP Classification 2016 by a pathologist. The slides were assessed under Olympus BX41 microscope. Selected pictures were taken using JENOPTIK SUBRA digital camera using the GRYPHAX software.

For evaluation of GATA3 immunohistochemical staining, the evaluation was done using the 400x magnification of the microscope. All the cases showing a visible brown staining for nucleus was taken as positive for GATA3 marker.

Appropriate scoring was done for the percentage of tumour cells and the staining intensity of tumour cells showing positive nuclear staining for GATA3 marker. The scoring for percentage of tumour cells stained by GATA3 was considered as: [6]

SCORE	**PERCENTAGE OF TUMOUR CELLS**
0	NO TUMOUR CELLS STAINED
1	1-10%
2	11-50%
3	51-80%
4	81-100%

The scoring for staining intensity of tumour cells stained by GATA3 was considered as: [6]

SCORE	**STAINING INTENSITY OF TUMOUR CELLS**
0	NO TUMOUR CELLS STAINED
1	WEAK
2	MODERATE
3	STRONG

The above scores obtained for percentage of tumour cells and staining intensity of tumour cells for GATA3 were then multiplied in order to calculate the immunoreactivity score for GATA3 staining. Based on the immunoreactivity scoring obtained, these cases were divided in to 4 groups: [6]

GROUPS	IMMUNOREACTIVITY SCORE	INTERPRETATION
I	0-1	NEGATIVE
II	2-4	WEAKLY POSITIVE
III	5-8	MODERATELY POSITIVE
IV	9-12	STRONGLY POSITIVE

Data analysis: Data obtained was entered in Microsoft Excel software and analyzed and expressed in percentages and proportions. . The association between variables was achieved using Chi-square test using SPSS (Statistical Package for Social Sciences) software version 20.0. A probability value (p) of ≤0.05 at 95% CI was considered statistically significant.

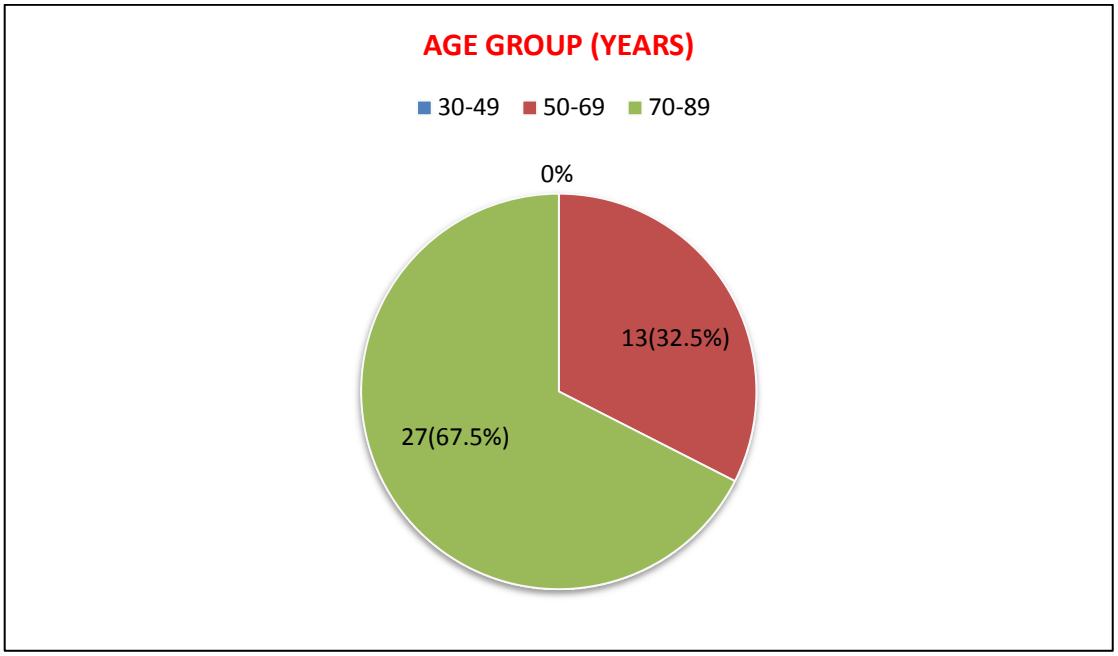
RESULTS

Data was collected for the 40 urothelial carcinoma cases and 40 prostate adenocarcinoma cases.

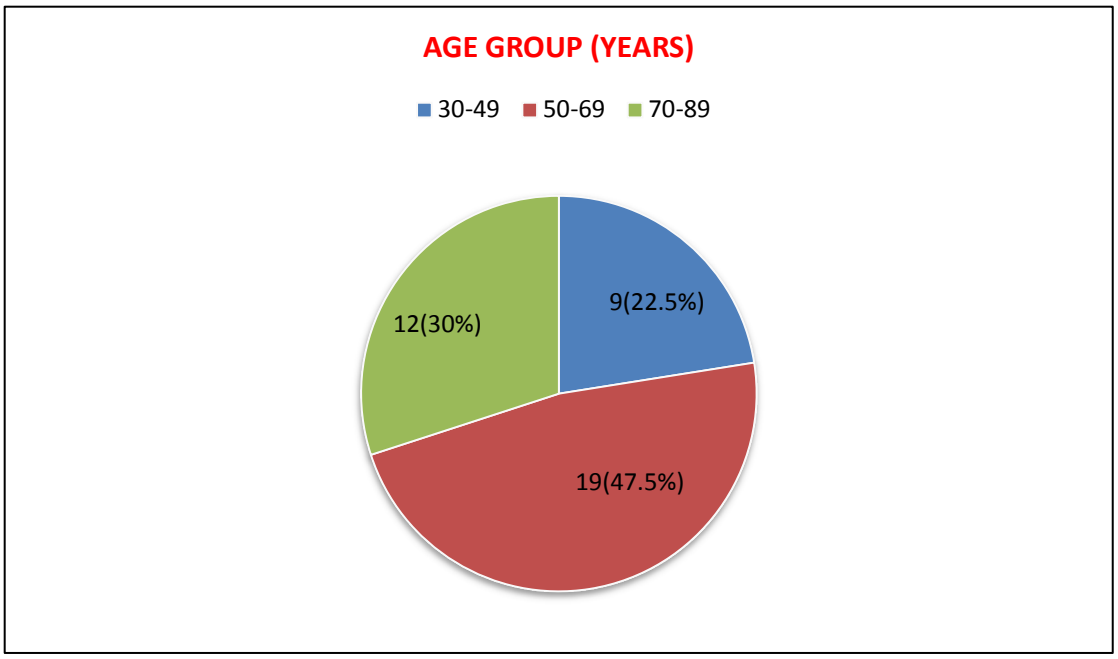
The data obtained from this study was compiled and tabulated. It was then statistically analysed.

The patient's age group ranged from 30 years - 87 years (Mean = 58.5 years) for the urothelial carcinoma cases and from 50 years - 85 years (Mean = 67.5 years) for the prostate adenocarcinoma cases.

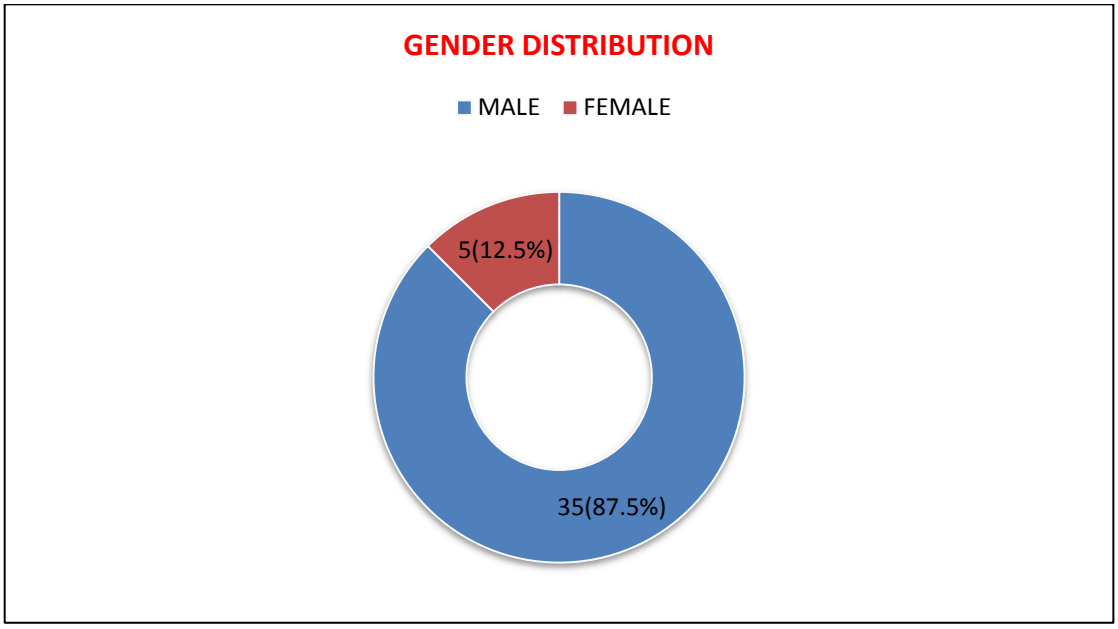
Graph 1: Age distribution among patients with prostate adenocarcinoma (n=40):



Graph 2: Age distribution among patients with urothelial carcinoma (n=40):



Graph 3: Gender distribution among urothelial carcinoma cases:



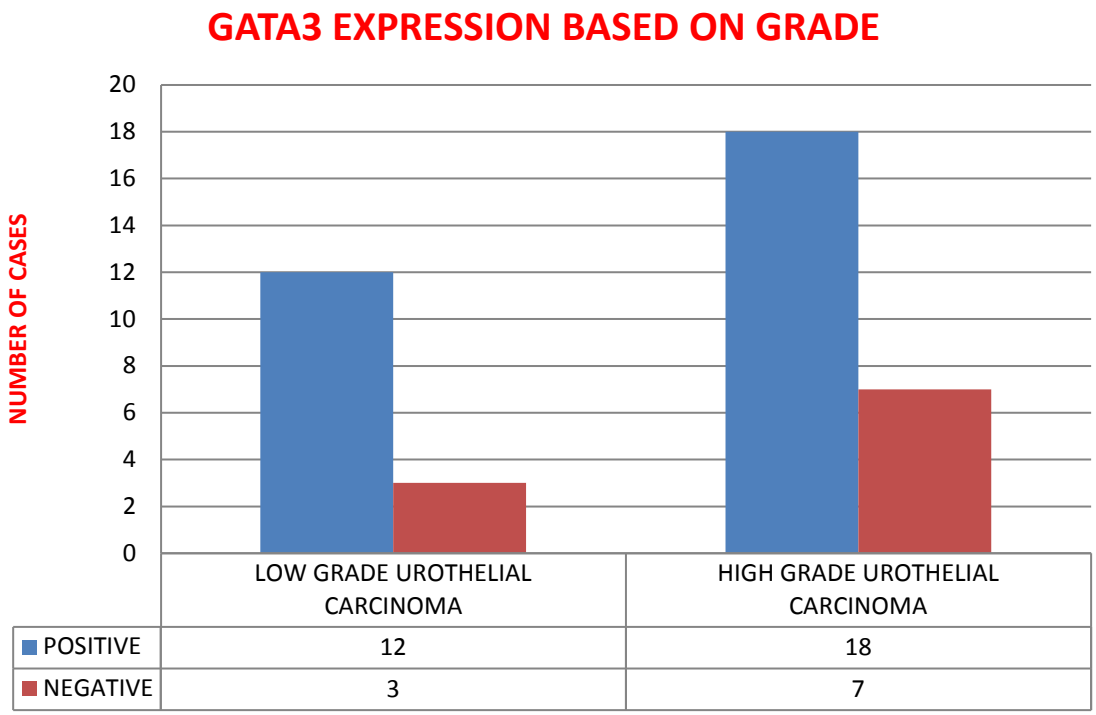
Among urothelial carcinoma cases, 35 cases were male patients while 5 cases were female patients.

Table 3: Test statistics:

GATA3 STAINING	UROTHELIAL CARCINOMA	PROSTATE ADENOCARCINOMA	TOTAL
POSITIVE	30	0	30
NEGATIVE	10	40	50
TOTAL	40	40	80

The GATA3 IHC marker stained positive for 30/40 (75%) and negative for 10/40 (25%) cases of urothelial carcinoma. The GATA3 IHC marker stained negative for all 40/40 (100%) cases of prostate adenocarcinoma in the study (p value = 0.0001)

Graph 4: GATA3 expression based on grade of urothelial carcinoma:



Out of the 15 cases of low grade urothelial carcinoma (LGUC) cases in the study, the GATA3 marker was positive in 12 (80%) cases and negative in 3 (20%) cases.

Out of the 25 cases of high grade urothelial carcinoma (HGUC) cases in the study, the GATA3 marker was positive in 18 (72%) cases and negative in 7 (28%) cases.

Table 4: Percentage of tumour cells stained by GATA3 in urothelial carcinoma:

SCORE	PERCENTAGE OF TUMOUR CELLS	NUMBER OF CASES
0	NO TUMOUR CELLS STAINED	10
1	1-10	2
2	11-50	8
3	51-80	09
4	81-100	11
TOTAL		40

It was observed that 9/40 (22.5%) cases and 11/40 (27.5%) cases showed 51-80% and 81-100% of tumour cells positive for GATA3 respectively. No tumour cells were stained in 10/40 (25%) cases in the study.

Table 5: Staining intensity of tumour cells for GATA3 in urothelial carcinoma:

SCORE	STAINING INTENSITY	NUMBER OF CASES
0	NO TUMOUR CELLS STAINED	10
1	WEAK STAINING	2
2	MODERATE STAINING	11
3	STRONG STAINING	17
TOTAL		40

It was observed that 28/40 (70%) cases showed moderate – strong staining for the GATA3 marker. However, 10/40 (25%) cases stained negative for the GATA3 marker.

Table 6: Categorizing the urothelial carcinoma cases based on the immunoreactivity score and its interpretation:

GROUPS	Gata3 EXPRESSION	IMMUNOREACTIVITY SCORE	CASES	PERCENTAGE
GROUP I	NEGATIVE	0-1	10	25
GROUP II	WEAK POSITIVITY	2-4	10	25
GROUP III	MODERATE POSITIVITY	5-8	4	10
GROUP IV	STRONG POSITIVITY	9-12	16	40
TOTAL			40	100

The urothelial carcinoma cases were then grouped based on the immunoreactivity score. 16/40 (40%) cases gave a score of 9-12 and were considered showing strong positivity for the marker. 4/40 (10%) cases and 10/40 (25%) cases gave a score of 5-8 (moderate positivity) and 2-4 (weak positivity) respectively. However, 10/40 (25%) cases gave a score of 0-1 and these were considered to be negative for GATA3 marker.

Graph 5: Pie chart showing percentage distribution of cases into different groups based on immunoreactivity score:

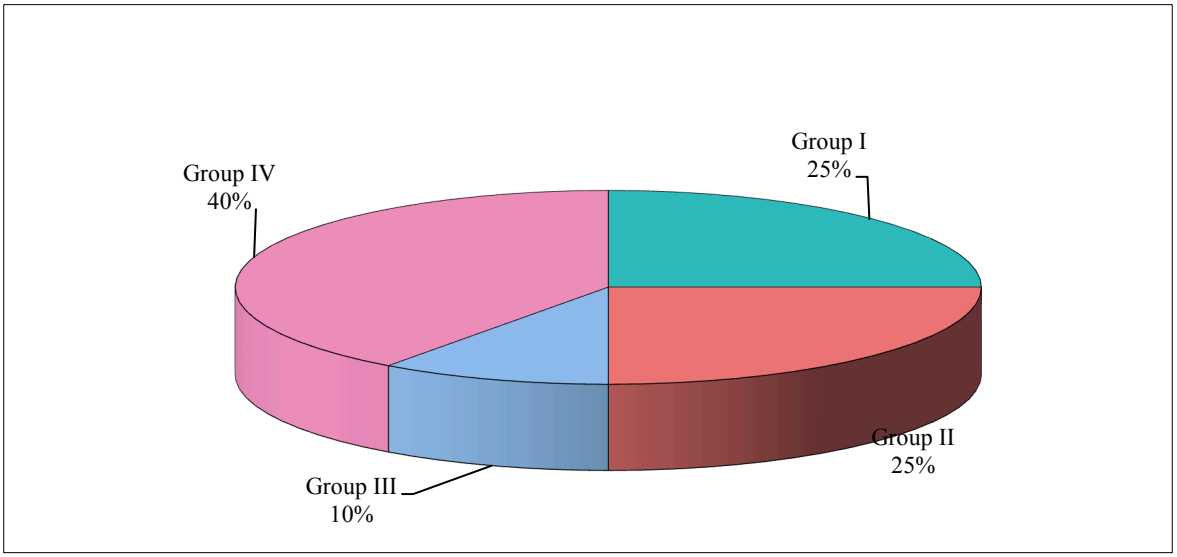


Table 7: Distribution of urothelial carcinoma patients based on personal habits and clinical presentation:

CLINICAL PRESENTATION (n =40)	No of patients	Percentage
Hematuria	37	92.5
Pain	17	42.5
Increased frequency	9	22.5
Obsrtuctive symptoms	9	22.5
PERSONAL HABITS (n = 40)	**No of patients**	**Percentage**
None	11	27.5
Smoker	11	27.5
Tobacco	7	17.5
Smoker + Tobacco	11	27.5

The symptom found commonly among the study population with urothelial carcinoma was hematuria seen in 37 (92.5%) patients in the study. Also, it was observed that 22 (55%) patients in the study had the habit of smoking which is a major risk factor that leads to bladder cancer.

Graph 6: Column chart showing case distribution based on clinical presentation and personal habits:

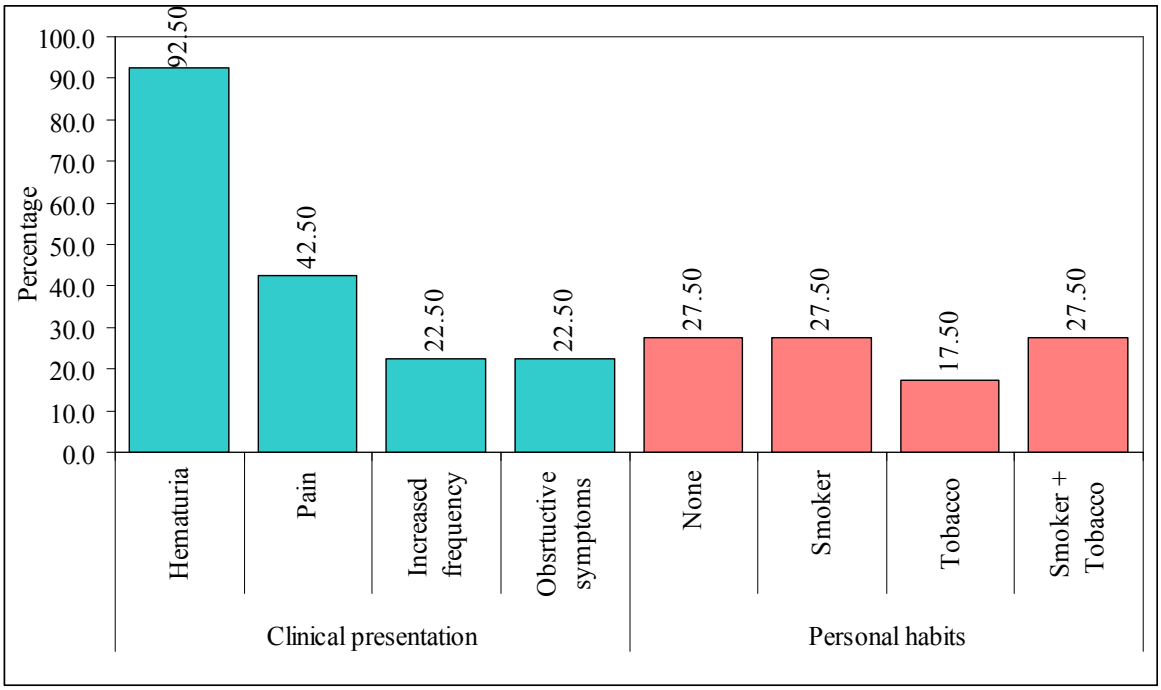


Table 8: Distribution of urothelial carcinoma patients based on site and size of tumour:

SITES (n = 40)	No of patients	Percentage
Bladder neck	3	7.5
Lateral wall	25	62.5
Others	12	30
TUMOUR SIZE (n = 40)		
<=3cm	24	60
>3cm	16	40

Among 40 urothelial carcinoma patients, the commonest site of involvement was the lateral wall by urothelial carcinoma and was seen in 25 (62.5%) cases. Considering the distribution of cases based on tumour size, 24 (60%) cases had tumour size <=3 cms and 16 (40%) cases had tumour size > 3cms.

Graph 7: Column chart showing case distribution based on site and size of tumour:

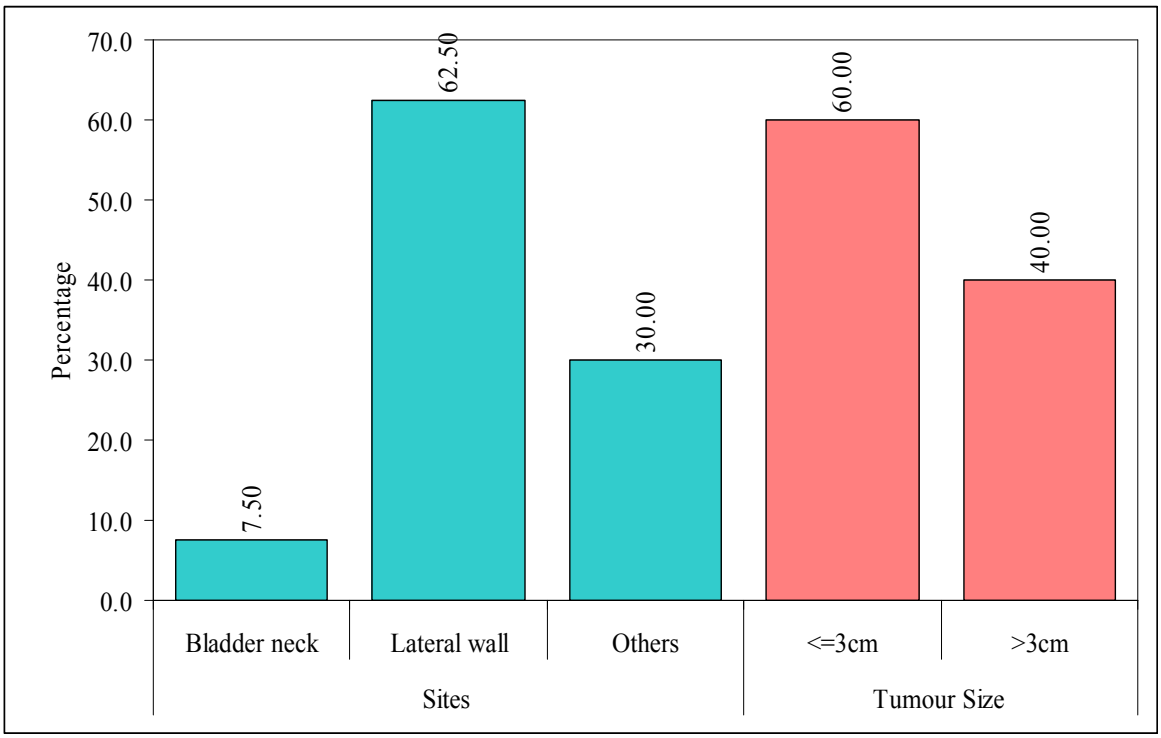
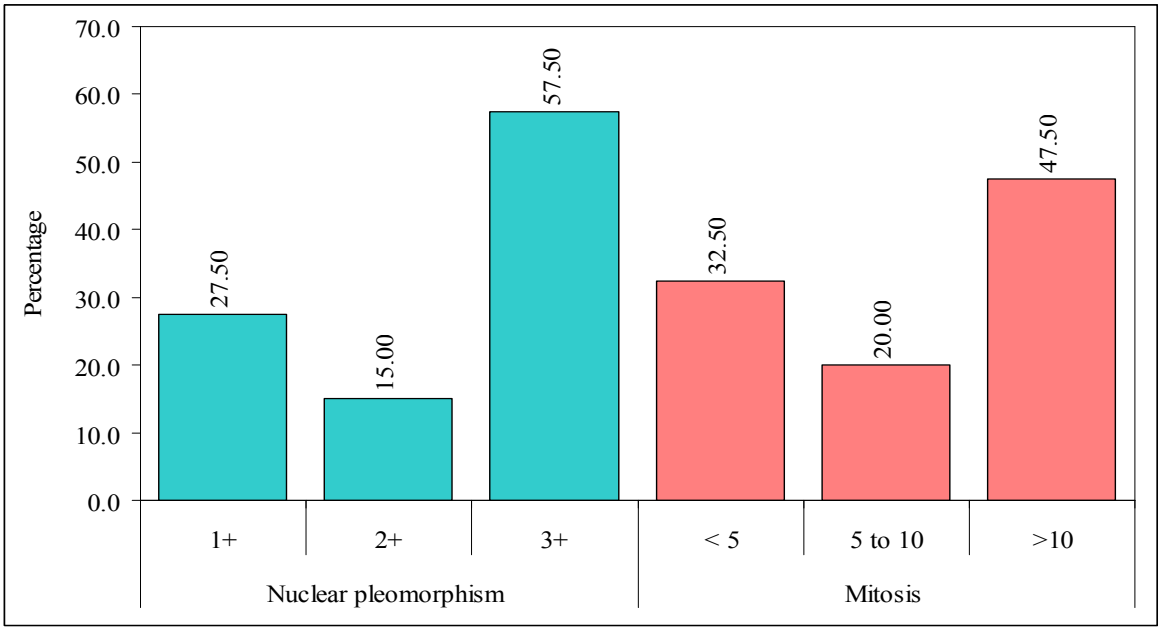


Table 9: Distribution of urothelial carcinoma patients based on pathological parameters:

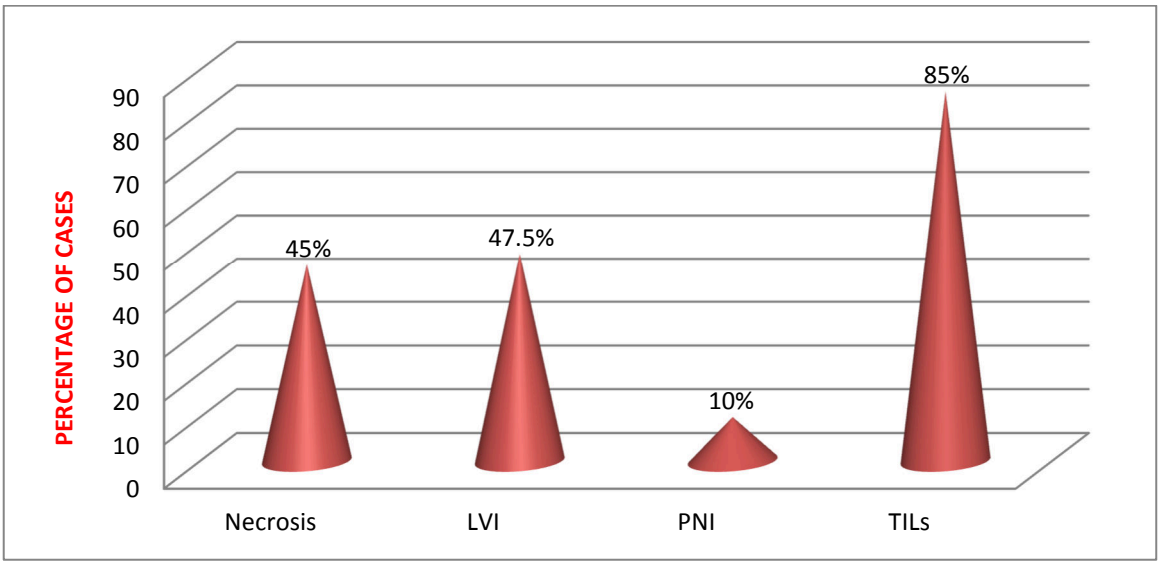
NUCLEAR PLEOMORPHISM (n = 40)	**No of patients**	**Percentage**
1+	11	27.5
2+	6	15
3+	23	57.5
MITOSIS (n = 40)		
< 5	13	32.5
5 to 10	8	20
>10	19	47.5
NECROSIS (n = 40)		
PRESENT	18	45
ABSENT	22	55
LYMPHOVASCULAR INVASION (LVI) (n = 40)		
PRESENT	19	47.5
ABSENT	21	52.5
PERINEURAL INVASION (PNI) (n = 40)		
PRESENT	4	10
ABSENT	36	90
TUMOUR INFILTRATING LYMPHOCYTES (TILs) (n = 40)		
PRESENT	34	85
ABSENT	6	15

Graph 8: Column chart showing case distribution based on nuclear pleomorphism and mitosis:



Among 40 cases of urothelial carcinoma, high nuclear pleomorphism (3+) and mitosis (>10/10HPF) were found in 23/40 (57.5%) cases and 19/40 (47.5%) cases in the study respectively.

Graph 9: Graph showing percentage of cases showing presence of Necrosis, PNI, LVI & TILs:



Out of the 40 cases with urothelial carcinoma, the percentage of cases in the study that showed features of necrosis, perineural invasion (PNI), lymphovascular invasion (LVI) and tumour infiltrating lymphocytes (TILs) were 45%, 10%, 47.5% and 85% of cases respectively.

Table 10: Comparison of groups with age groups:

Age groups	Grp I	%	Grp II	%	Grp III	%	Grp IV	%	Total	%
30-49	4	40	1	10	1	25	3	18.75	9	22.5
50-69	3	30	6	60	2	50	8	50	19	47.5
>=70	3	30	3	30	1	25	5	31.25	12	30
Mean age	59.10		64.80		62.50		61.88		61.98	
SD age	17.10		13.52		17.79		15.04		15.01	
Total	10	100	10	100	4	100	16	100	40	100
Chi-square=3.21, p=0.78										

Among the 40 urothelial carcinoma cases studied, group I and II had mean age 59 and 65 years respectively. The group III and IV had mean age 63 and 62 years respectively. The p-value was 0.78 which was not statistically significant. No statistically significant association between expression of GATA3 and age groups seen.

Graph 10: Comparison of groups with age groups:

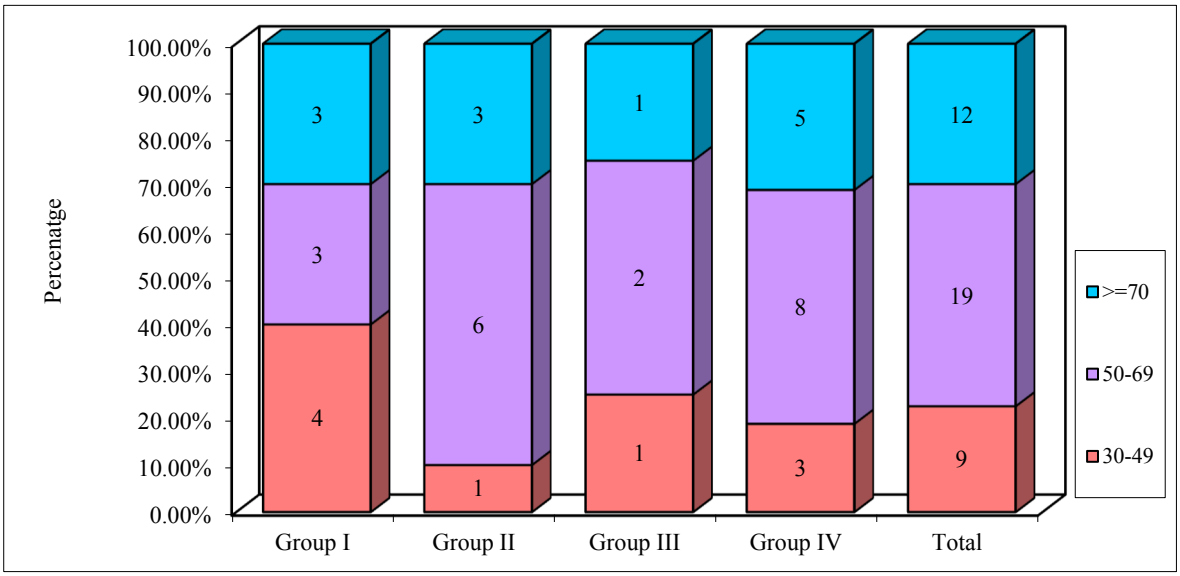


Table 11: Comparison of groups with gender:

Gender	Group I	%	Group II	%	Group III	%	Group IV	%	Total	%
Male	9	90	8	80	4	100	14	87.5	35	87.5
Female	1	10	2	20	0	0	2	12.5	5	12.5
Total	10	100	10	100	4	100	16	100	40	100
Chi-square=1.14, p=0.77										

Among the 40 cases of urothelial carcinoma studied, group I had 1 female and 9 males while the group IV had 2 females and 14 males. The p- value was 0.77. No statistically significant association between GATA3 expression and gender observed.

Graph 11: Comparison of groups with gender:

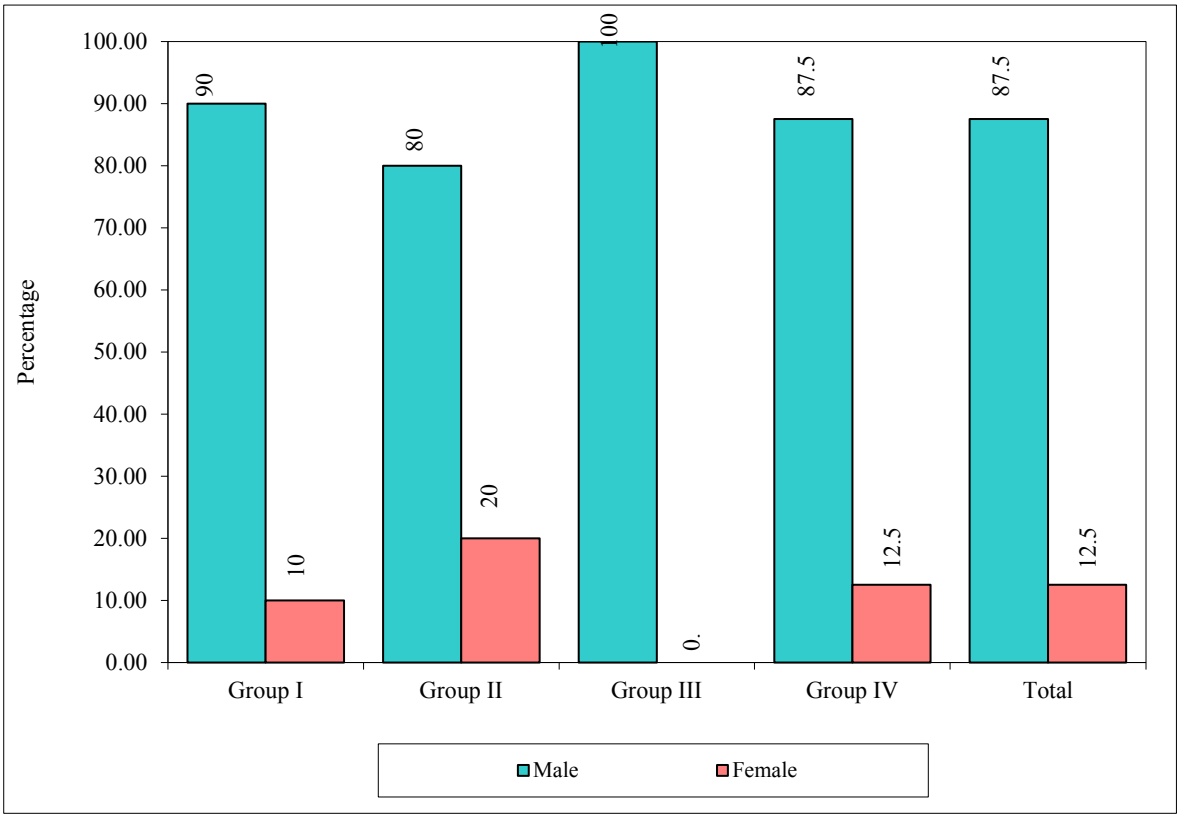


Table 12: Comparison of groups with tumour grades:

Grades	Group I	%	Group II	%	Group III	%	Group IV	%	Total	%
HGUC	7	70	9	90	1	25	8	50	25	62.5
LGUC	3	30	1	10	3	75	8	50	15	37.5
Total	10	100	10	100	4	100	16	100	40	100
Chi-square=6.93, p=0.07										

Among the 40 cases studied, it was observed that group I and group II had 7 HGUC 3 LGUC and 9 HGUC 1LGUC cases respectively and the group III and group IV had 1 HGUC 3 LGUC and 8 HGUC 8 LGUC cases respectively. The p value = 0.07 and therefore, there was no statistically significant association between the tumour grade and GATA3 expression in the study.

Graph 12: Comparison of groups with tumour grades:

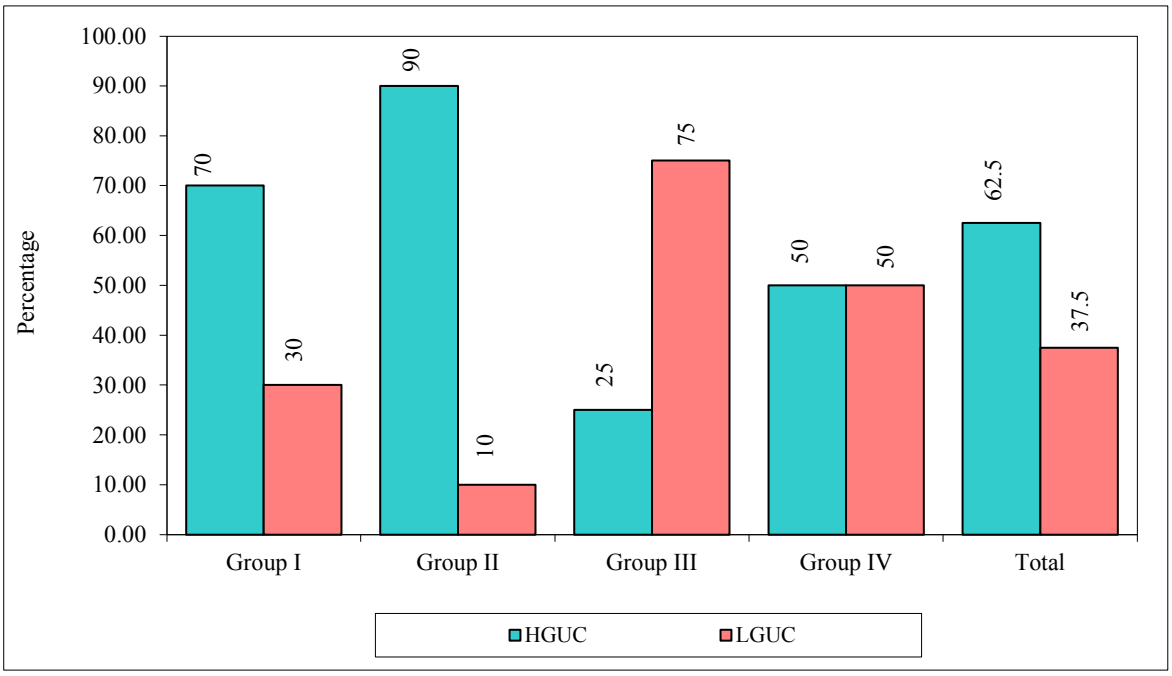


Table 13: Comparison of groups with nuclear pleomorphism:

Nuclear pleomorphism	Group I	%	Group II	%	Group III	%	Group IV	%	Total	%
1+	2	20	1	10	1	25	7	43.75	11	27.5
2+	1	10	2	20	2	50	1	6.25	6	15
3+	7	70	7	70	1	25	8	50	23	57.5
Total	10	100	10	100	4	100	16	100	40	100
Chi-square=8.72, p=0.19										

In the 40 cases studied, high nuclear pleomorphism (3+) was found in 7 (70%), 7 (70%), 1 (25%) and 8 (50%) cases of urothelial carcinoma in group I, group II, group III and group IV respectively. The p value was 0.19. No statistically significant association found between GATA3 expression and nuclear pleomorphism in the study.

Graph 13: Comparison of groups with nuclear pleomorphism:

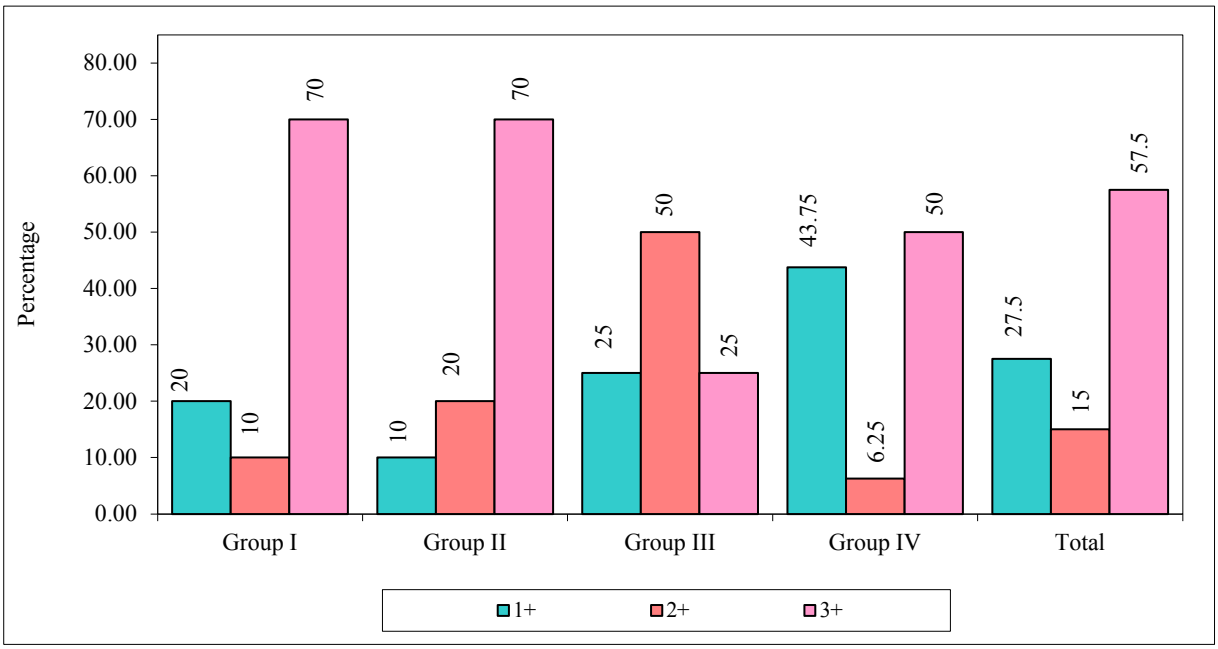


Table 14: Comparison of groups with mitosis:

Mitosis (/10HPF)	Group I	%	Group II	%	Group III	%	Group IV	%	Total	%
< 5	3	30	2	20	2	50	6	37.5	13	32.5
5 to 10	0	0	2	20	1	25	5	31.25	8	20
>10	7	70	6	60	1	25	5	31.25	19	47.5
Total	10	100	10	100	4	100	16	100	40	100
Chi-square=6.77, p=0.34										

In the 40 cases studied, high degree of mitosis (>10/10HPF) was found in 7 (70%), 6 (60%), 1 (25%) and 5 (31.25%) urothelial carcinoma cases in group I, group II, group III and group IV respectively. The p value was 0.34. No statistically significant association between mitosis and GATA3 expression observed in the study.

Graph 14: Comparison of groups with mitosis:

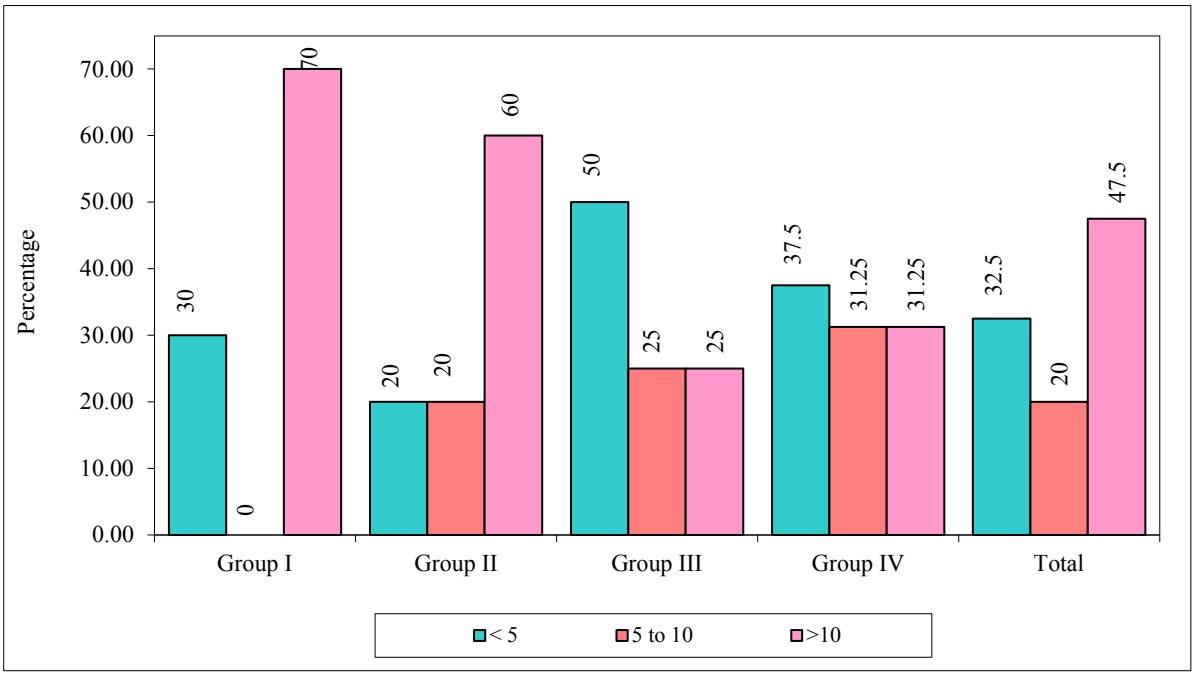


Table 15: Comparison of groups with the presence of Necrosis, PNI, LVI and TILs:

	Group I	%	Group II	%	Group III	%	Group IV	%	Total	%	p-value
Necrosis	4	40	5	50	2	50	7	43.75	18	45	0.97
LVI	4	40	8	80	1	25	6	37.5	19	47.5	0.12
PNI	1	10	2	20	0	0	1	6.25	4	10	0.61
TIL	9	90	9	90	3	75	13	81.25	34	85	0.83

Among 40 cases of urothelial carcinoma, the cases that showed the presence of Necrosis, PNI, LVI and TILs were further classified under group I, group II, group III and group IV based on GATA3 expression and tabulated as above (Table 15). The p values were calculated as 0.97, 0.61, 0.12 and 0.83 for the presence of Necrosis, PNI, LVI and TILs respectively. The association between these 4 pathological parameters and expression of GATA3 marker was not statistically significant.

Graph 15: Comparison of groups with the presence of Necrosis, PNI, LVI and TILs:

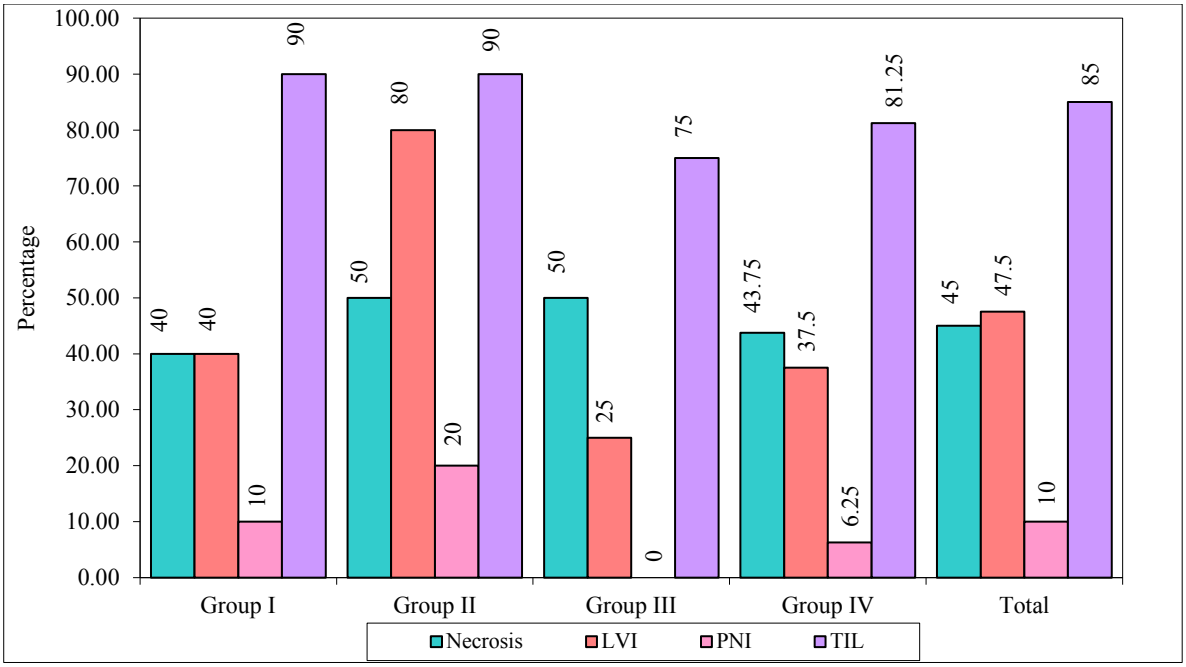


Table 16: Comparison of groups with site of tumour:

Sites	Group I	%	Group II	%	Group III	%	Group IV	%	Total	%
Bladder neck	2	20	0	0	1	25	0	0	3	7.5
Lateral wall	3	30	6	60	3	75	13	81.25	25	62.5
Others	5	50	4	40	0	0	3	18.75	12	30
Total	10	100	10	100	4	100	16	100	40	100
Chi-square=11.91, p=0.06										

Among the 40 urothelial carcinoma cases, the lateral wall was the site frequently involved for primary bladder cancer. Involvement of lateral wall was seen in 3 (30%), 6 (60%), 3 (75%) and 13 (81.25%) cases in group I, group II, group III and group IV respectively. The p value =0.06. No statistically significant correlation between the site of tumour and GATA3 expression observed.

Graph 16: Comparison of groups with site of tumour:

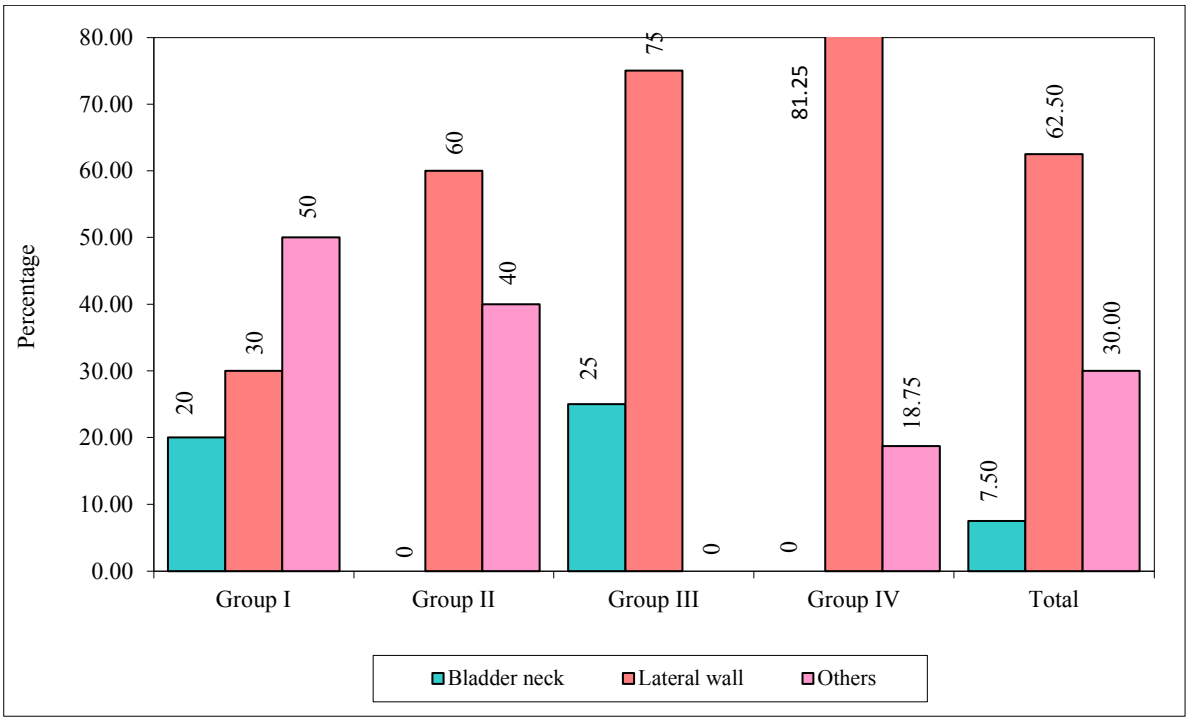


Table 17: Comparison of groups with clinical features:

Symptoms	Grp I	%	Grp II	%	Grp III	%	Grp IV	%	Total	%
Hematuria	9	90	10	100	4	100	14	87.5	37	92.5
Pain	4	40	6	60	0	0	7	43.75	17	42.5
Increased frequency	1	10	3	30	0	0	5	31.25	9	22.5
Obstructive symptoms	1	14.29	3	30	0	0	5	26.32	9	22.5

Hematuria was most common symptom found among the study group (92.5%). Out of the 40 urothelial carcinoma cases, hematuria was found in 9 (90%), 10 (100%), 4 (100%) and 14 (87.5%) cases in group I, group II, group III and group IV respectively. No statistically significant association between clinical features and GATA3 presentation seen.

Graph 17: Comparison of groups with clinical features:

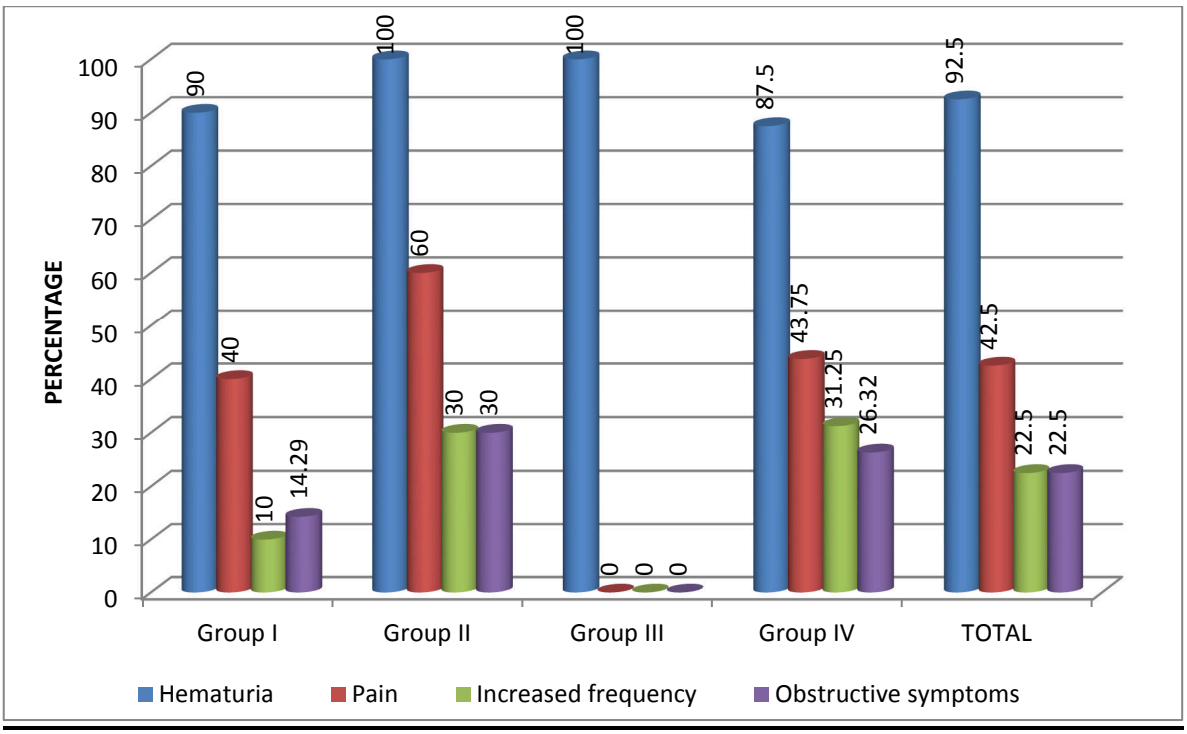


Table 18: Comparison of groups with personal habits:

Personal Habits	Group I	%	Group II	%	Group III	%	Group IV	%	Total	%
None	3	30	1	10	1	25	6	37.5	11	27.5
Smoker	1	10	3	30	1	25	6	37.5	11	27.5
Tobacco	2	20	1	10	0	0	4	25	7	17.5
Smoker + Tobacco	4	40	5	50	2	50.	0	0	11	27.5
Total	10	100	10	100	4	100	16	100	40	100
Chi-square=12.57, p=0.18										

The personal habits of the 40 patients were compared with the 4 groups based on GATA3 expression. The p value = 0.18 and hence no statistically significant correlation between personal habits and GATA3 expression.

Graph 18: Comparison of groups with personal habits:

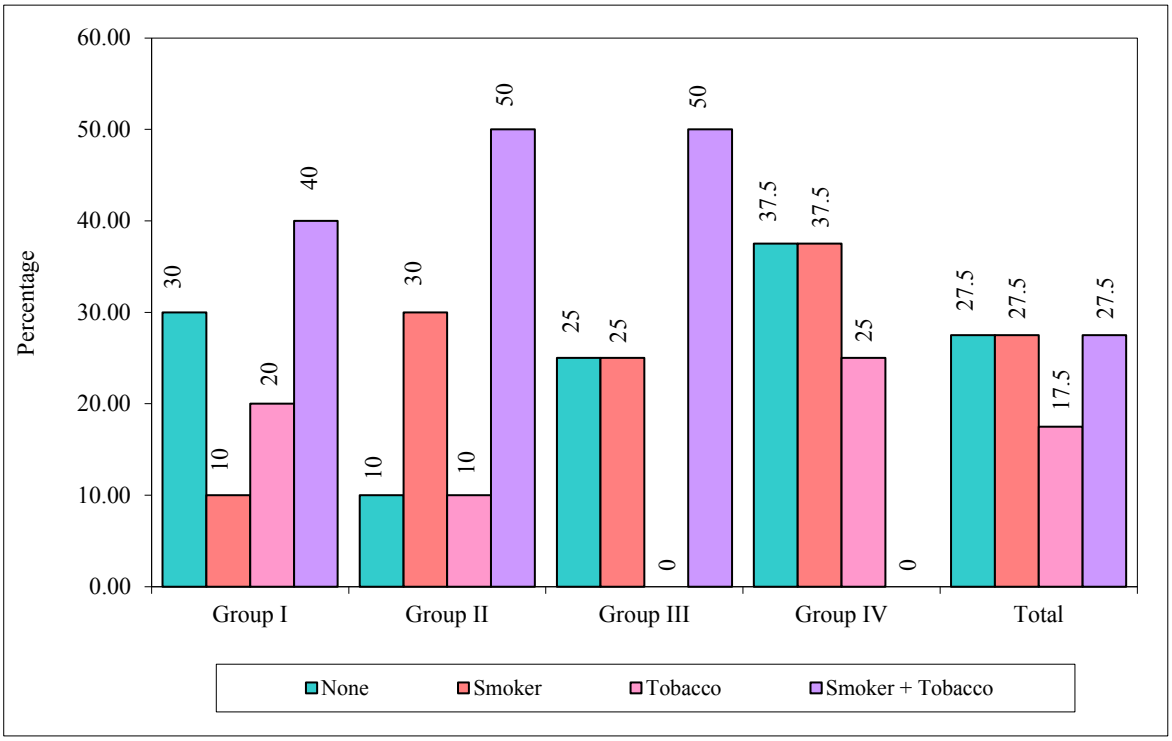
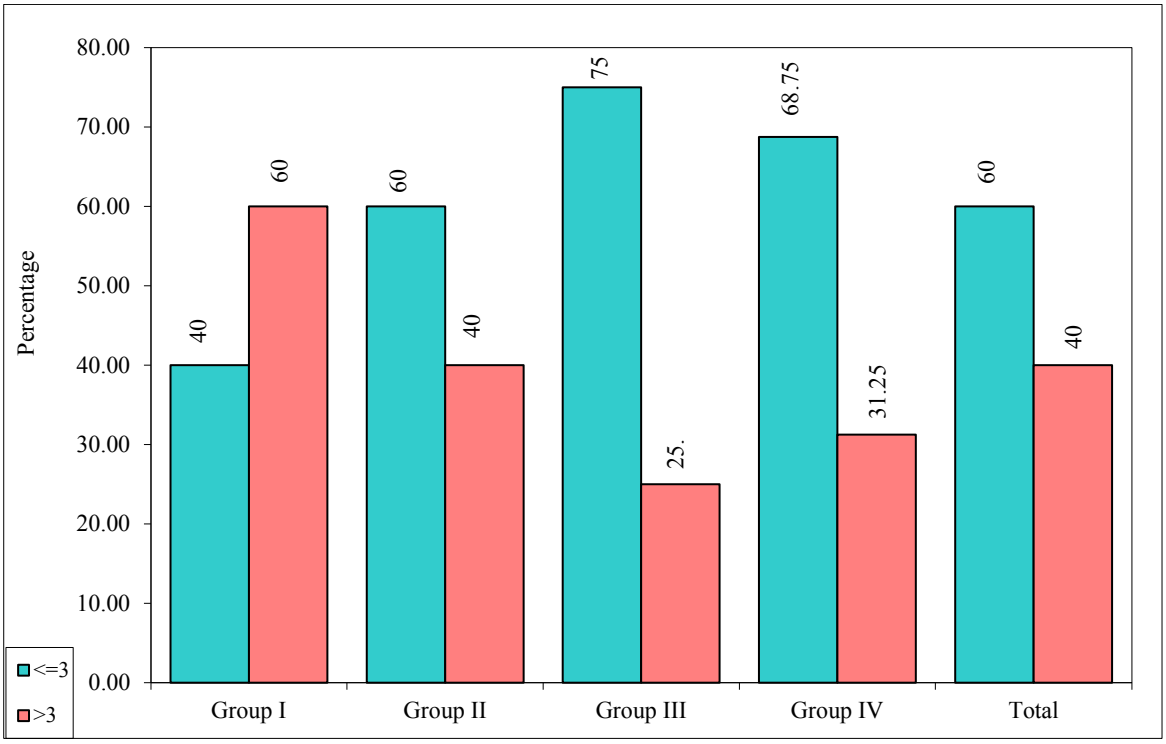


Table 19: Comparison of groups with tumour size:

Tumour size (cm)	Group I	%	Group II	%	Group III	%	Group IV	%	Total	%
<=3	4	40	6	60	3	75	11	68.75	24	60
>3	6	60	4	40	1	25	5	31.25	16	40
Total	10	100	10	100	4	100	16	100	40	100
Chi-square=2.55, p=0.47										

The tumour size of the 40 urothelial carcinomas cases were divided into 2 groups: <=3 and >3 cms. These groups based on size were then compared with the 4 groups based on GATA3 expression and was tabulated (Table 19). The p value was calculated to be 0.47. Hence, no statistically significant correlation between the tumour size and GATA3 expression was seen.

Graph 19: Comparison of groups with tumour size:



DISCUSSION

Bladder cancer ranks 10th among the most frequently diagnosed cancers and prostate cancer ranks 2nd among the most frequently diagnosed cancer in men worldwide as per the GLOBOCAN 2018.[1] There is an increase in the five year prevalence rate for both bladder and prostate cancer from 3.21% to 3.57% and from 6.78% to 9.47% respectively between the years 2018 and 2020.[2, 3]

Bladder cancers are seen commonly in men when compared to women while prostate adenocarcinomas occur in men only. Prostatic adenocarcinoma is an important differential diagnosis to be ruled out while assessing a case of urothelial carcinoma as both the prostate and urinary bladder lie close to each other in their anatomical location. The prostate maybe involved by infiltrating urothelial carcinoma either by direct invasion into the stroma of prostate or by intraductal extension with or without the invasion into prostate stroma. Also, the bladder may be involved by prostate adenocarcinoma by metastasis or through direct extension to the bladder.[7]

The histopathological diagnosis of prostate adenocarcinoma and urothelial carcinoma is usually not difficult using routine hematoxylin and eosin staining technique. However, it becomes a challenge in cases of poorly differentiated adenocarcinoma of prostate particularly those arising from the bladder neck when the histopathological and clinical features are similar to infiltrating high grade urothelial carcinoma.[4] This makes it difficult to differentiate a high grade prostate adenocarcinoma extending in to urinary bladder from a high grade urothelial carcinoma infiltrating the prostate. This is relatively a common diagnostic dilemma when transurethral resection specimens are received to distinct both the entities. [6]

It is necessary to differentiate both these carcinomas as their treatment modalities, staging and prognosis differ.[6] A cystoprostatectomy usually considered as a surgical procedure to treat an infiltrating high grade bladder carcinoma cannot be considered for the treatment and staging of prostate carcinoma. Therefore, when the histological features of both bladder and prostate carcinomas overlap, it becomes necessary to resort to use of immunohistochemical markers to solve this diagnostic challenge so that the appropriate treatment can be decided for the patient.[4, 5]

Many markers have been studied to understand whether the poorly differentiated tumour has a prostatic or urothelial origin. One such marker most recently recognized is the GATA3 marker which is a urothelial associated immunohistochemical marker which is found to aid in the differentiation of urothelial carcinoma and prostate adenocarcinoma.[6]

GATA is a type of transcription factors which attach to the DNA sequence [A/T] GATA [A/G] located in the gene promoters and is directly involved in the expression of target genes by their activation or repression.[115] It was in 2007, Higgins *et al*. first studied the utility of GATA3 expression as a marker to recognize urothelial carcinoma and found the marker specific for urothelial carcinoma.[116] Few of the studies that studied GATA 3 IHC expression in various tumours concluded that GATA3 marker is a sensitive for cancers of the breast and urothelial carcinoma. [43, 117]

Our study was done to evaluate the use of GATA 3 immunohistochemical marker to differentiate urothelial carcinoma from prostate adenocarcinomas. The study evaluated a total of 80 cases among which 40 cases were urothelial carcinoma and 40 cases were prostate adenocarcinoma.

Table 20: Comparing mean age for urothelial carcinoma with other studies

	Present study	**Abdelmaksoud AA et al [4]**	**Abdullah WH et al [5]**	**Agarwal H et al [6]**
Mean age for urothelial carcinoma	58.5 years	59 years	70.2 years	55.9 years

In the present study, mean age for urothelial carcinoma was 58.5 years. The patients age for urothelial carcinoma ranged from 30 years to 87 years. Commonest age group was 50-69 years with 19 cases (47.5%).

When compared to the Abdelmaksoud AA et al [4] and Abdullah WH et al [5] studies, the mean age for urothelial carcinoma were 59 years and 70.2 years respectively. In the study done by Agarwal H et al [6], who considered 74 patients of urothelial carcinoma, mean age was 55.9 years and most of their cases – 45 cases (60.81%) were in age group 40 – 60 years.

Among the cases of prostate adenocarcinoma, the mean age in our study was 67.5 years. The patients age for prostate adenocarcinoma ranged from 50 years to 85 years. Commonest age group was 70-89 years with 27 cases (67.5%). When compared to the Abdelmaksoud AA et al [4] and Abdullah WH et al [5] studies, the mean age for prostate adenocarcinoma were 63.5 years and 72.3 years respectively.

Table 21: Comparing GATA3 expression in urothelial carcinoma with other studies

Studies	Number of cases showing positivity	Percentage of cases showing positivity
Present study	30/40	75%
Agarwal H et al [6]	57/74	77%
Abdelmaksoud AA et al [4]	25/30	83.3%
Abdullah WH et al [5]	49/51	96%
Chang A et al [117]	28/35	80%
Mohammed KH et al [9]	56/79	70.8%

Various studies show that in urothelial carcinomas, the GATA 3 marker exhibits a variable expression and ranges from 63% to 96%. The GATA3 expression for urothelial carcinoma cases in our study was 75% (30/40). In the studies done by Agarwal H et al [6], Abdelmaksoud AA et al [4] and Abdullah WH et al [5], the GATA 3 positivity was observed to be 77% (57/74), 83.3% (25/30) and 96% (49/51). Chang A et al [117] and Mohammed KH et al [9] also showed a positivity of 80% (28/35) and 70.8% (56/79) for GATA3 marker respectively.

Table 22: Comparing GATA3 expression in prostate adenocarcinoma with other studies

Studies	Number of cases showing positivity	Percentage of cases showing positivity
Present study	0/40	0%
Agarwal H et al [6]	0/10	0%
Abdelmaksoud AA et al [4]	0/30	0%
Abdullah WH et al [5]	0/15	0%
Chang A et al [117]	0/38	0%
Mohammed KH et al [9]	0/48	0%

In the study done by Chang A *et al.*[117] in 2012 and Agarwal H *et al.*[6] in 2016, they demonstrated that the prostate adenocarcinoma showed no GATA3 immunoexpression. In our study also, similar results were observed as all the 40 prostate adenocarcinoma cases stained negative for GATA3 marker suggesting the specificity of the marker for urothelial carcinoma. Similar findings were also seen in the studies done by Abdelmaksoud AA et al [4], Abdullah WH et al [5] and Mohammed KH et al.[9]

Hence, GATA 3 marker can be considered in the routine IHC panel used to assess whether the origin of a tumour is urothelium or prostate. It has also been observed that they express the same immunostaining properties at both primary and metastatic sites.[118]

In the present study, GATA 3 expression of urothelial carcinoma was compared with various clinical parameters like the age, gender, personal habits, clinical presentation and site of tumour. Out of the 40 urothelial carcinoma cases studied, 35 were men and 5 were women and the M - F ratio was 7:1. Smoking was the common habit in the urothelial carcinoma patients. Hematuria was more common symptom in the study population. The urothelial carcinomas were commonly seen involving lateral wall of bladder among the cases studied. No association between positivity of GATA3 and above parameters were observed unlike the study by Agarwal H *et al.*[6] which stated that the incidence of hematuria was high in those cases which showed GATA 3 expression. However, this was not appreciated in other studies done so far.

Table 23: Comparing GATA3 expression with grade of tumour

	Present study	**Agarwal H et al [6]**	**Miyamoto et al [115]**	**Abdullah WH et al [5]**
Comparison of GATA3 with grade of tumour	10 cases negative 7/10 (70%) HGUC 3/10 (30%) LGUC	14 cases negative 14/14 (100%) HGUC 0/14 (0%) LGUC	20 cases negative 19/20 (95%) HGUC 1/20 (5%) LGUC	2 cases negative 2/2 (100%) HGUC 0/2 (0%) LGUC

In the study done by Miyamoto *et al.* [115] and Agarwal H *et al.*[6], they demonstrated that there is statistically significant correlation between histological grade of the tumour with GATA 3 expression in urothelial carcinoma. Miyamoto *et al.* [115] in their study demonstrated that loss of expression of GATA3 marker was correlating with high grade of tumour but its strong expression independently predicts poor prognosis of the tumour. Out of the 20 negative cases in study done by Miyamoto *et al.* [115], 19/20 (95%) cases were HGUC and 1/20 (5%) cases were LGUC. Out of the 14 negative cases in study done by Agarwal H *et al* [6], 14/14 (100%) cases were HGUC and 0/14 (0%) cases were LGUC.

In our study with 10 negative cases, we had similar findings with 7/10 (70%) HGUC cases and 3/10 (30%) LGUC cases. However, no statistically significant association between histological grade and GATA 3 expression was observed. This is supported by the study done Abdullah WH *et al* [5] which also had 2/2(100%) cases of HGUC and 0/2 (0%) cases of LGUC among the negative cases but no significant association between the grade of tumour and expression of GATA3 was observed.

In a study done by Mohammed KH *et al* [9] it was found that expression of GATA3 was high in those invasive urothelial carcinomas cases which had larger tumour size but no such association was seen in any other studies including our study.

In our study, the association between expression of GATA3 and other histopathological parameters like nuclear pleomorphism, mitosis, necrosis, LVI, PNI and TILs were also studied. No statistically significant correlation between these histopathological parameters and GATA 3 expression was seen. The only study that demonstrated statistically significant correlation between these parameters and GATA 3 expression was the study done by Agarwal H *et al* [6], where they observed reduced or absent expression of GATA 3 marker was associated with increased nuclear pleomorphism, increased mitosis (> 10 mitosis/10HPF), presence of TILs and necrosis. However, such associations were not observed in our study as well as other studies.

LIMITATIONS OF PRESENT STUDY:

1. Sample size was limited. A larger sample size is required to generalize the findings of the study.
2. Low sample size could be the reason for not getting statistically significant coorelation between expression of GATA3 and tumour grade.
3. Majority of the urothelial carcinoma samples were TURBT specimens. Hence, expression of GATA3 could not be correlated with clinicopathological parameters like tumour stage and muscle invasion by tumour.

CONCLUSION

The findings of this study highlight the significance of using the immunohistochemical marker GATA3 to determine whether the origin of poorly differentiated tumour is from the bladder or prostate in situations where one finds it difficult to differentiate between an infiltrating high grade urothelial carcinoma involving the prostate and a high grade prostate adenocarcinoma involving the bladder.

This differentiation is important as it will guide in giving the correct diagnosis and avoiding any misdiagnosis which will help in proper staging of the tumour and selecting the appropriate treatment and management for the patient.

GATA 3 immunohistochemical marker showed a positivity of 75% among the urothelial carcinoma cases in this study while none of the prostate adenocarcinoma cases stained for the marker. This suggests GATA 3 immunostain as a valuable marker to be included in the IHC panel for urothelial carcinomas to diagnose a primary tumour of bladder or a carcinoma of the bladder that metastasize and involve other sites, along with the help of clinical history and morphological findings of the tumour.

It was also observed that GATA3 marker was more negative among high grade urothelial carcinomas (7/10) compared to low grade urothelial carcinomas (3/10). However, our study concluded there was no statistically significant association between the expressions of GATA 3 marker with any clinicopathological parameters like age, gender, personal habits, clinical presentation, site of tumour, tumour grade, tumour size, nuclear pleomorphism, mitosis, necrosis, LVI, PNI and TILs.

SUMMARY

- This cross-sectional study was done at the surgical pathology laboratory in KLE'S DR. PRABHAKAR KORE HOSPITAL and MEDICAL RESEARCH CENTER, BELAGAVI by collecting data and blocks of urothelial carcinoma and prostate adenocarcinoma cases between January 2018 and December 2020.
- The objectives of the study were to evaluate the role of GATA3 immunohistochemical marker to differentiate between urothelial carcinoma and prostatic adenocarcinoma and to correlate GATA3 positivity score with clinicopathological parameters.
- 40 specimens of urothelial carcinoma and 40 specimens of prostate adenocarcinoma were considered in the study
- Among urothelial carcinoma cases, 35 patients were male and 5 patients were female showing that urothelial carcinoma are common among men than women.
- The study revealed 30/40 (75%) cases of urothelial carcinoma staining positive for the GATA3 immunohistochemical marker and all the prostate adenocarcinoma cases stained negative for the marker showing high specificity of the marker to urothelial carcinoma.
- Hence, GATA 3 immunohistochemical marker is valuable marker that needs to be included in the IHC panel to differentiate high grade urothelial carcinoma from high grade prostate adenocarcinoma.
- Among different histological grades of urothelial carcinoma, 12/15 (80%) cases of low grade carcinoma and 21/25 (84%) cases of high grade carcinoma stained positive for the GATA3 marker.

- It was observed that out of the 10 cases which were negative, 3/10 (30%) cases were low grade while 7/10 (70%) were high grade urothelial carcinomas. Higher the grade of tumour, there was increased chance of becoming negative for the GATA3 marker. However, no statistically significant association between expression of GATA3 marker and histological grade of the tumour was observed.
- No statistically significant correlation between other clinicopathological parameters like age, gender, personal habits, clinical presentation, site of tumour, tumour size, mitosis, nuclear pleomorphism, necrosis, LVI, PNI and TILs with the expression of GATA3 marker was seen.

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ANNEXURE-I

WHO CLASSIFICATION OF UROTHELIAL TRACT TUMOURS, 2016, 4th Edition

1. **UROTHELIAL TUMOURS**

   A. **Infiltrating urothelial carcinoma**

   - 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

   B. **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
   -

2. **SQUAMOUS CELL NEOPLASMS**

   - Pure squamous cell carcinoma
   - Verrucous carcinoma
   - Squamous cell papilloma

3. **GLANDULAR NEOPLASMS**

    - Adenocarcinoma, NOS
        - ✓ Enteric
        - ✓ Mucinous
        - ✓ Mixed
    - Villous adenoma

4. **URACHAL CARCINOMA**

5. **TUMOURS OF MÜLLERIAN TYPE**

    - Clear cell carcinoma
    - Endometrioid carcinoma

6. **NEUROENDOCRINE TUMOURS**

    - Small cell neuroendocrine carcinoma
    - Large cell neuroendocrine carcinoma
    - Well differentiated neuroendocrine tumour
    - Paraganglioma

7. **MELANOCYTIC TUMOURS**

    - Malignant melanoma
    - Nevus
    - Melanosis

8. **MESENCHYMAL TUMOURS**

    - Rhabdomyosarcoma
    - Leiomyosarcoma
    - Angiosarcoma
    - Inflammatory myofibroblastic tumour
    - Perivascular epithelioid cell tumour
        - o Benign
        - o Malignant

- Solitary fibrous tumour
- Leiomyoma
- Hemangioma
- Granular cell tumour
- Neurofibroma

9. **UROTHELIAL TRACT HEMATOPOIETIC AND LYMPHOID TUMOURS**

10. **MISCELLANEOUS TUMOURS**

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

ANNEXURE-II

TNM CLASSIFICATION OF BLADDER CANCER, 2017, 8th Edition

TNM STAGE	FINDINGS
PRIMARY TUMOUR [T]	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Ta	Non-invasive papillary carcinoma
Tis	Urothelial Carcinoma in situ: “flat tumour”
T1	Tumour invades subepithelial connective tissue (lamina propria)
T2	Tumour invades muscularis propria
T2a	Tumour invades superficial muscularis propria (inner half)
T2b	Tumour invades deep muscularis propria (outer half)
T3	Tumour invades perivesical tissue
T3a	Microscopically
T3b	Macroscopically (extravesical mass)
T4	Extravesical tumour invades any of the following: prostatic stroma, seminal vesicles, uterus, vagina, pelvic wall, abdominal wall

T4a	Extravesical tumour invades prostatic stroma , seminal vesicles, uterus, vagina
T4b	Extravesical tumour invades pelvic wall, abdominal wall
REGIONAL LYMPH NODE (N)	
NX	Lymph node cannot be assessed
N0	No lymph node metastasis
N1	Single regional lymph node metastasis in the true pelvis (perivesical, obturator, internal and external iliac, or sacral lymph node)
N2	Multiple regional lymph node metastasis in the true pelvis (perivesical, obturator, internal and external iliac, or sacral lymph node metastasis)
N3	Lymph node metastasis to the common iliac lymph nodes
DISTANT METASTASIS (M)	
M0	No distant metastasis
M1	Distant metastasis
M1a	Distant metastasis limited to lymph nodes beyond the common iliacs
M1b	Non-lymph-node distant metastases

STAGE GROUPING:

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

ANNEXURE-III

WHO CLASSIFICATION OF PROSTATE TUMOURS, 2016, 4th Edition

1. EPITHELIAL TUMOURS:-

A. Glandular neoplasms

B. Acinar adenocarcinoma
   a) Atrophic
   b) Pseudohyperplastic
   c) Microcystic
   d) Foamy gland
   e) Mucinous (colloid)
   f) Signet ring like cell
   g) Pleomorphic giant cell
   h) Sarcomatoid

C. Prostatic intraepithelial neoplasm
   a) High grade

D. Intraductal carcinoma

E. Ductal adenocarcinoma
   a) Cribriform
   b) Papillary
   c) Solid

F. Urothelial carcinoma

G. Squamous neoplasm
   a) Adenosquamous carcinoma
   b) Squamous cell carcinoma

H. Basal cell carcinoma

2. NEUROENDOCRINE TUMOURS:-

a) Adenocarcinoma with neuroendocrine differentiation
b) Well differentiated neuroendocrine tumour
c) Small cell and large cell neuroendocrine neoplasm

3. MESENCHYMAL TUMOURS:-

a) Stromal tumour of uncertain malignant potential
b) Stromal sarcoma
c) Leiomyosarcoma
d) Rhabdomyosarcoma
e) Leiomyoma
f) Angiosarcoma
g) Synovial sarcoma
h) Inflammatory myofibroblastic tumour
i) Osteosarcoma
j) Undifferentiated pleomorphic sarcoma
k) Solitary fibrous tumour
l) Solitary fibrous tumour , malignant
m) Haemangioma
n) Granular cell tumour

4. HEMATOLYMPHOID TUMOURS:-

a) Diffuse large B-cell lymphoma
b) Chronic lymphocytic leukaemia/ small lymphocytic lymphoma
c) Follicular lymphoma
d) Mantle cell lymphoma
e) Acute myeloid leukaemia
f) B lymphoblastic leukaemia/lymphoma

5. MISCELLANEOUS TUMOURS

a) Cystadenoma
b) Nephroblastoma
c) Rhabdoid tumour
d) Germ cell tumour
e) Clear cell adenocarcinoma
f) Melanoma
g) Paraganglioma
h) Neuroblastoma

6. METASTATIC TUMOURS

ANNEXURE-IV

TNM CLASSIFICATION OF PROSTATE ADENOCARCINOMA, 2017, 8th Edition

TNM STAGE	FINDINGS
PRIMARY TUMOUR [T]	**CLINICAL**
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
T1	Clinically inapparent tumour neither palpable nor visible by imaging
T1a	Tumour incidental histological finding in 5 % or less of tissue resected
T1b	Tumour incidental histological finding in more than 5 % of tissue resected
T1c	Tumour identified by needle biopsy(e.g. Because of elevated PSA level)
T2	Tumour confined within prostate
T2a	Tumour involves one-half of one lobe or less
T2b	Tumour involves more than one-half of one lobe but not both lobes

T2c	Tumour involves both lobes
T3	Tumour extends through the prostate capsule
T3a	Extracapsular extension (unilateral or bilateral)
T3b	Tumour invades seminal vesicle(s)
T4	Tumour is fixed or invades adjacent structures other than seminal vesicle such as external sphincter, rectum, bladder, levator muscles and/ or pelvic wall
PRIMARY TUMOUR [pT]	**PATHOLOGICAL**
pT2	Organ confined
pT2a	Unilateral, one half of one side or less
pT2b	Unilateral, involving more than one-half of side but not both sides
pT2c	Bilateral disease
pT3	Extraprostatic extension
pT3a	Extraprostatic extension or microscopic invasion of bladder neck
pT3b	Seminal vesicle invasion
pT4	Invasion of rectum, levator muscles and/or pelvic wall

REGIONAL LYMPH NODE (N)	**CLINICAL**
NX	Regional lymph nodes were not assessed
N0	No regional lymph node metastasis
N1	Metastasis in regional lymph node(s)
REGIONAL LYMPH NODE (pN)	**PATHOLOGICAL**
pNX	Regional nodes not sampled
pN0	No positive regional node
pN1	Metastases in regional node(s)
DISTANT METASTASIS (M)	
M0	No distant metastasis
M1	Distant metastasis
M1a	Non regional lymph nodes
M1b	Bone(s)
M1c	Other site(s) with or without bone disease

STAGE GROUPING

Stage I	T1a-T2a	N0	M0
Stage II	T2b-c	N0	M0
Stage III	T3	N0	M0
Stage IV	T4	N0	M0
	Any T	N1	M0
	Any T	Any N	M1

ANNEXURE-V

INFORMED CONSENT FORM

EVALUATION OF ROLE OF GATA3 EXPRESSION IN DIFFERENTIATING UROTHELIAL CARCINOMAS FROM ADENOCARCINOMAS OF PROSTATE

Purpose of the study: The purpose of this study is to evaluate the utility of GATA3 expression in differentiating urothelial carcinomas from adenocarcinomas of prostate. You are being asked to enroll in this study as you are eligible for participation in this study. If you are diagnosed with urothelial carcinoma or prostatic adenocarcinoma, you will be included in this study.

Procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge.

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to know a better way to differentiate urothelial carcinoma from prostatic adenocarcinoma which is essential for providing appropriate treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KAHER University, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following person.

Principal Investigator: _____

Guide : _____

If you have any queries about your rights as a study subject, you may call Dr. Roopa Bellad, Professor, Department of Pediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No-9448113403, at J.N. Medical College, Belagavi.

Name of the participant: (signature/thumbprint)

Name of the witness: (signature/thumbprint)

Name of the investigator: (signature)

Date:

Address:

Phone no:

ANNEXURE-VI

ETHICAL CLEARANCE CERTIFICATE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed to-be-University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 261 **Date: 24/12/2019**

To,
Reg.no: BN0119004
PG student in Pathology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EVALUATION OF ROLE OF GATA3 EXPRESSION IN DIFFERENTIATING UROTHELIAL CARCINOMAS FROM ADENOCARCINOMAS OF PROSTATE"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Anita Dalal)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURES VII – PROFORMA

PROFORMA FOR UROTHELIAL CARCINOMA

<table>
<tr><td colspan="2">NAME:</td><td>AGE:</td><td>SEX:</td><td>IP NO:</td><td>SAMPLE NO:</td></tr>
<tr><td colspan="6">CLINICAL HISTORY:</td></tr>
<tr><td colspan="6">CLINICAL DIAGNOSIS:</td></tr>
<tr><td colspan="6">OPERATIVE PROCEDURE:</td></tr>
<tr><td colspan="3">TUMOUR SIZE:</td><td colspan="3">TUMOUR SITE:</td></tr>
<tr><td colspan="6">HISTOPATHOLOGICAL DIAGNOSIS:</td></tr>
<tr><td colspan="2">GRADE:</td><td colspan="3">NUCLEAR PLEOMORPHISM:</td><td>MITOSIS:</td></tr>
<tr><td>NECROSIS:</td><td colspan="2">LVI:</td><td colspan="2">PNI:</td><td>TILs:</td></tr>
<tr><td colspan="6">IHC STAINING:</td></tr>
<tr><td colspan="6">PERCENTAGE OF TUMOUR CELLS STAINED:</td></tr>
<tr><td colspan="6">STAINING INTENSITY:</td></tr>
<tr><td colspan="6">IMMUNOREACTIVITY SCORE:</td></tr>
<tr><td colspan="6">INTERPRETATION:</td></tr>
</table>

PROFORMA FOR PROSTATE ADENOCARCINOMA

NAME:	**AGE:**	**SEX:**	**IP NO:**	**SAMPLE**
CLINICAL HISTORY:				**PSA :**
CLINICAL DIAGNOSIS:				
OPERATIVE PROCEDURE:				
HISTOPATHOLOGICAL DIAGNOSIS:				
GLEASON SCORE:		**GROUP GRADE:**		
PERINEURAL INVASION:				
IHC STAINING:				
PERCENTAGE OF TUMOUR CELLS STAINED:				
STAINING INTENSITY:				
IMMUNOREACTIVITY SCORE:				
INTERPRETATION:				

ANNEXURE-VIII

HEMATOXYLIN AND EOSIN STAINING PROTOCOL

1. Deparaffinize in Xylene I and II and III changes. (III change use warmed xylene) (5 minutes in each)
2. Rehydrate using
    a. Absolute ethanol 100% (5 minutes)
    b. Absolute Ethanol 100% (5 minutes)
3. Rinse in distilled water (5 minutes)
4. Rinse in running tap water (5 minutes)
5. Stain in Harris's haematoxylin by progressive method (2 minutes) Fresh and filtered
6. Rinse in running tap water (20 minutes)
7. Decolorize in 1% acid alcohol (1 second)
8. Rinse well in tap water (5 minutes)
9. Immerse in hot water bath, 55˚C for blueing (3 seconds)
10. Rinse in tap water (5 minutes)
11. Counterstain in Eosin (15 seconds)
12. Dehydrate with absolute alcohol 100% (2-4 dips)
13. Clear in xylene I and II (5 minutes)
14. Mount with DPX.

Stock solution – Eosin:

Stock – 1% aqueous Eosin – Y

Stock – 1% aqueous Phloxin B

Working Solution – Eosin:

100ml stock Eosin

10 ml stock Phloxin B

780 ml 95% Ethanol

4 ml glacial acetic acid

Working Solution – Hematoxylin

Harris Hematoxylin, 1 litre

Working solution – 0.25% Acid alcohol

95% Ethanol, 2578 ml

dH2O, 950 ml

HCl, 9ml

Result: Nuclei – blue, cytoplasm – pink, RBCs – red.

Reference: Bancroft D, Laylon C. The haematoxylin and eosin, In: Kim SS Ed, Bancroft's Theory and practice of histopathological techniques. &th Ed., China, Churchill Livingstone; 2013: p173-187.

PROCEDURE FOR IHC STAINING FOR GATA3 ANTIBODY

1. Cut the sections at approximately 3-4 μm thickness in poly L Lysine coated slides.
2. Float on to the positive charged slides.
3. Slides were air dried for 2 hours at 58 ºC.
4. Two changes of xylene of 10 minutes each for deparaffinization.
5. Hydration:
    a) Absolute alcohol - 2 dips
    b) 80% alcohol - 2 dips
    c) 70% alcohol - 2 dips
    d) Distilled water - 2 changes 5 minutes each.
6. Antigen retrieval by heat, using microwave and TRIS EDTA Buffer.
7. Cooling of sections to room temperature.
8. Rinse in distilled water for 3 minutes.
9. Wash in Tris buffered saline **(**TBS) buffer two times for 3 minutes each.
10. Treatment with peroxide block for 10 minutes to block endogenous peroxidase.
11. Wash in TBS buffer two times for 3 minutes each.
12. Treatment with primary antibody (GATA3) for 60 minutes
13. Wash in TBS buffer two times for 3 minutes each
14. Treatment with Target binder for 10 minutes
15. Wash in TBS buffer two times for 3 minutes each
16. Treatment with HRP Polymer for 10 minutes
17. Wash in TBS buffer two times for 3 minutes each
18. Treatment with DAB (secondary antibody) for 3-5 minutes to give brown colour to antigens

19. Wash in distilled water for 3 minutes
20. Counter stain with Harris haematoxylin for 30 seconds to 1 minute
21. Wash in tap water for 3 minutes to remove excess stain
22. Two changes of absolute alcohol for 2 minutes each for dehydration
23. Clearing with xylene for two minutes. Dry the slides and mount with DPX

PREPARATION OF REAGENTS

1. Antigen retrieval Buffer

TRIS EDTA Buffer- pH: 8.5 to 9.0

Preparation:

TRIS Base- 1.21 gram

EDTA (atomic number: 372)- 0.37 grams

Dissolve in 1000ml of water

2. Wash buffer

TRIS BUFFERED SALINE (TBS)- pH: 7.2 to 7.6

Preparation:

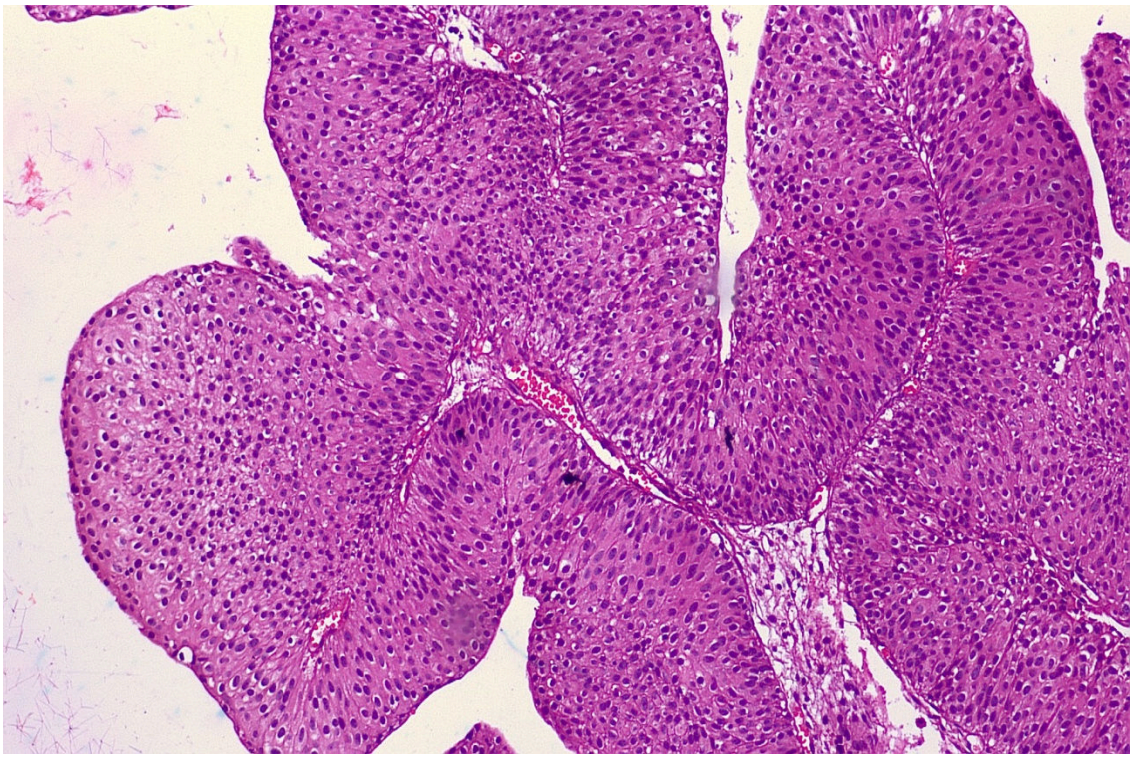
TRIS Base- 8.6 gram

NaCl- 9.6 gram

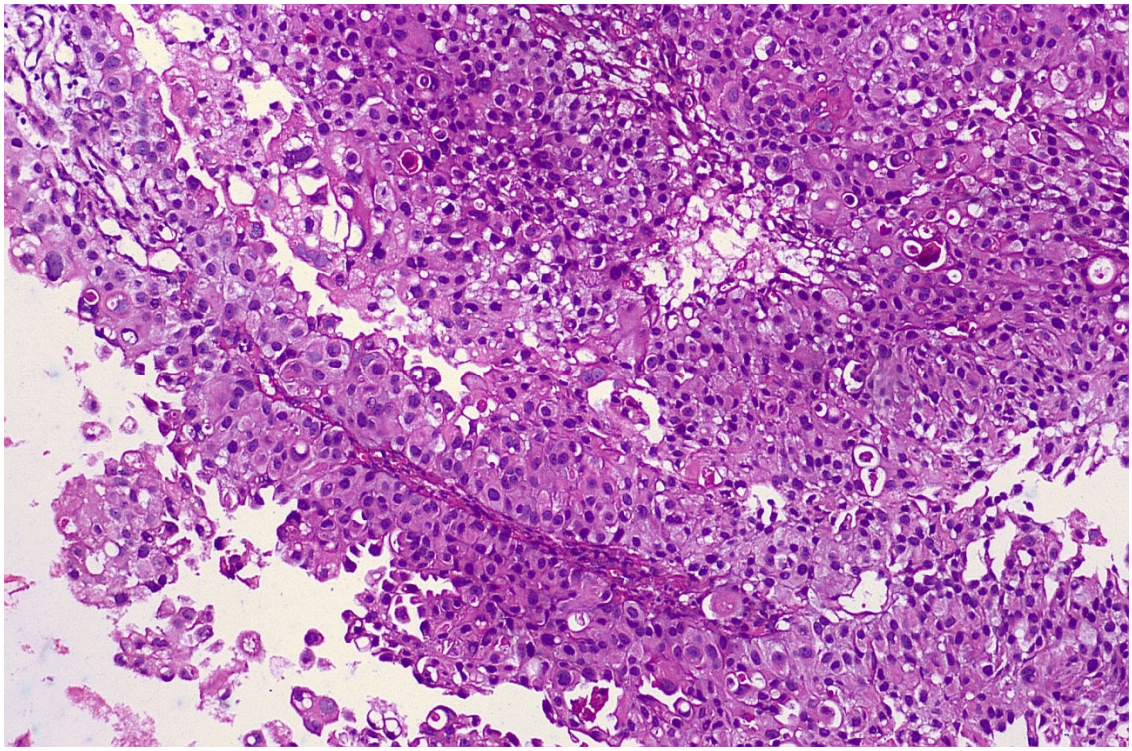
Dissolve in 1000ml of water.

Adjust pH by using concentrated HCl

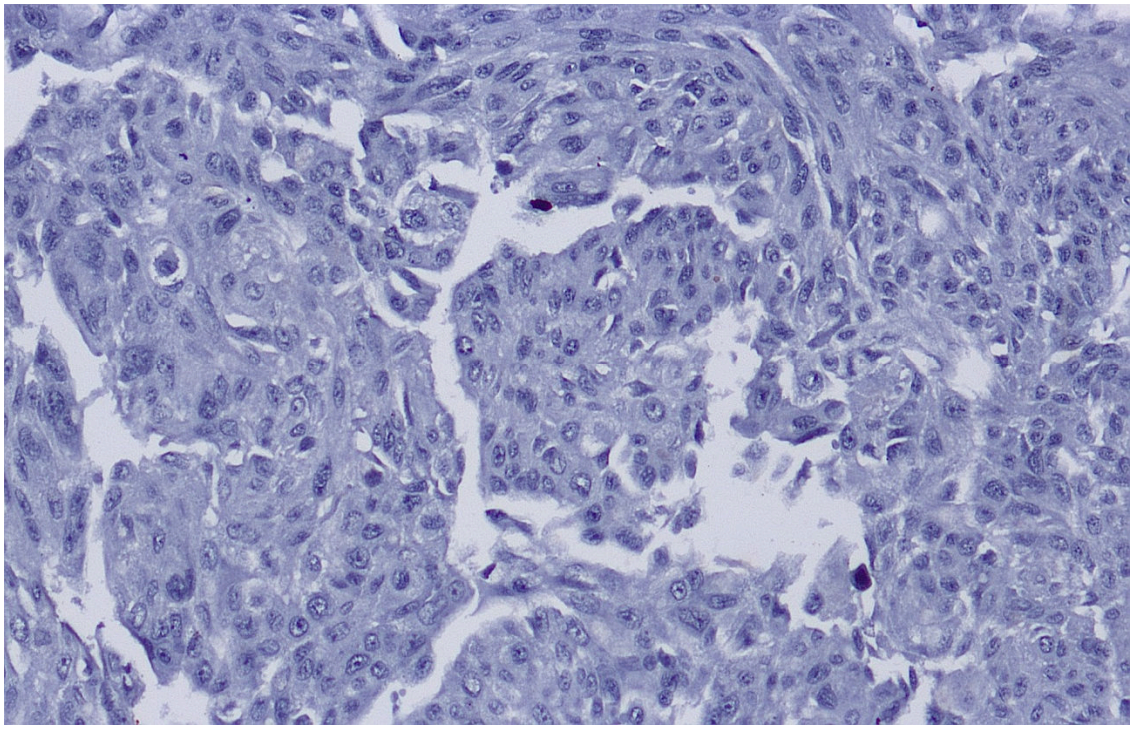
ANNEXURE IX– PHOTOMICROGRAPHS



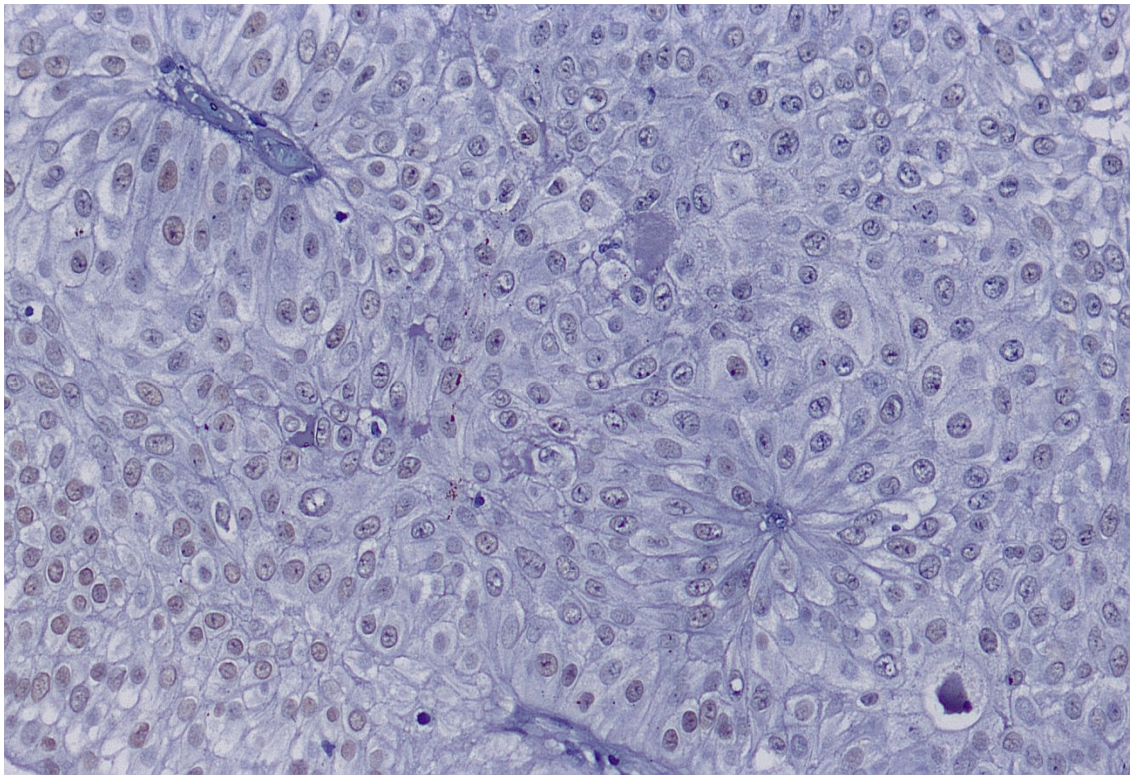
Photomicrograph 1: Low Grade Urothelial carcinoma (H&E- 20X).



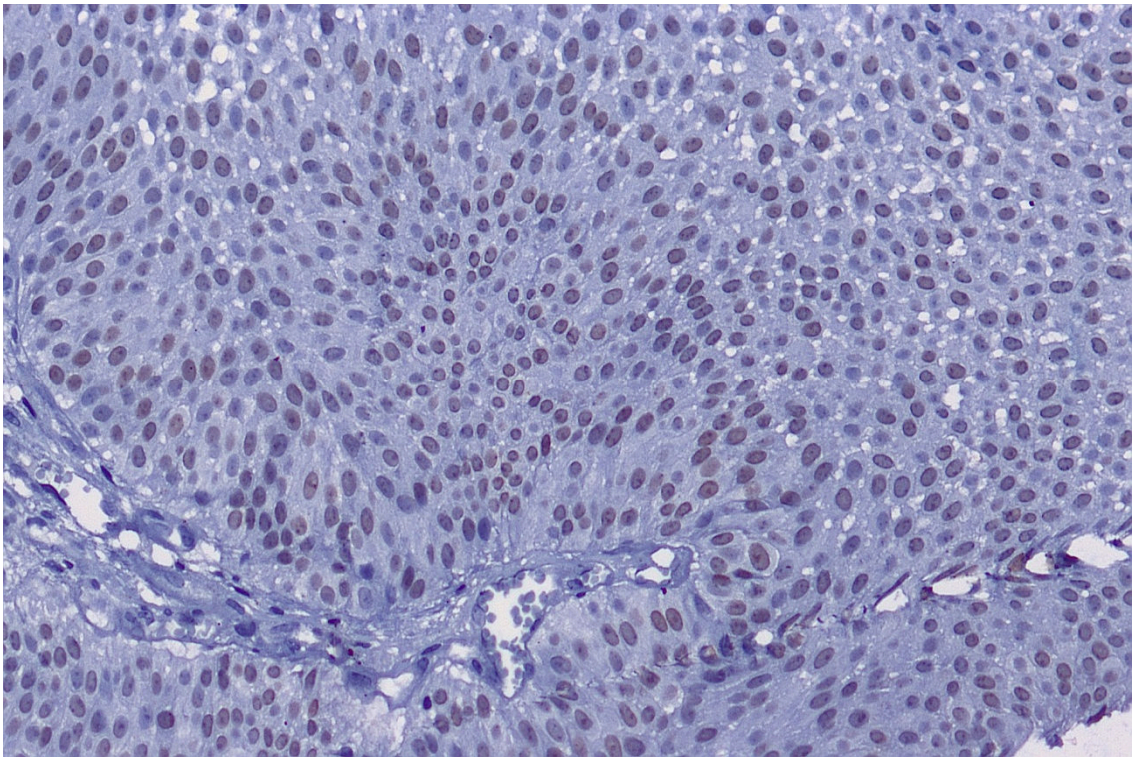
Photomicrograph 2: High Grade Urothelial carcinoma (H&E- 20X).



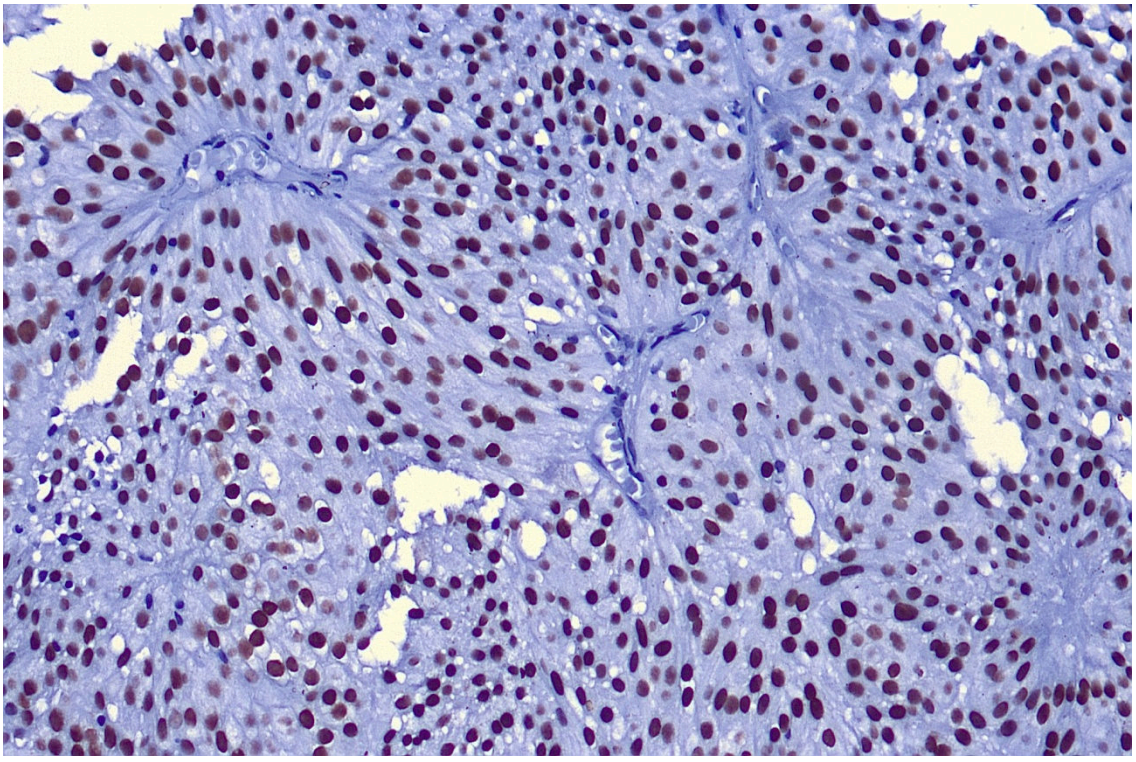
Photomicrograph 3: Group I – No brown nuclear staining. Negative for GATA3 marker (IHC- 40X)



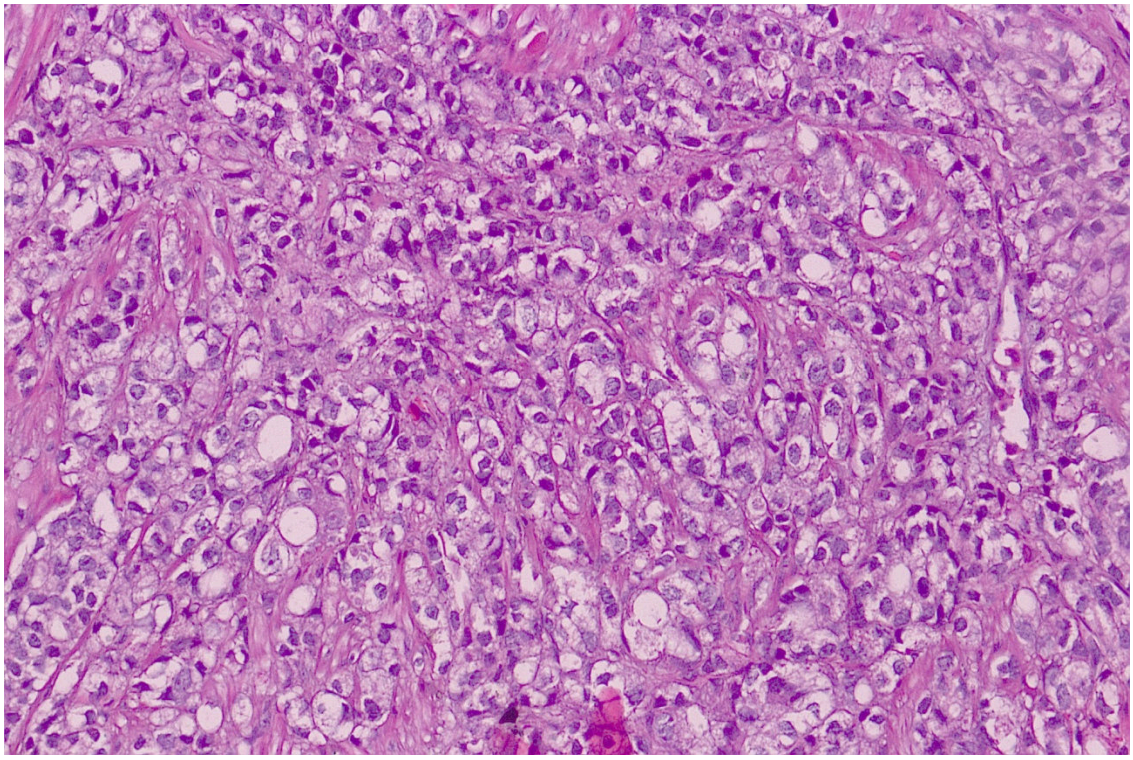
Photomicrograph 4: Group II – The nucleus staining light brown colour. Weakly positive for GATA3 marker (IHC- 40X)



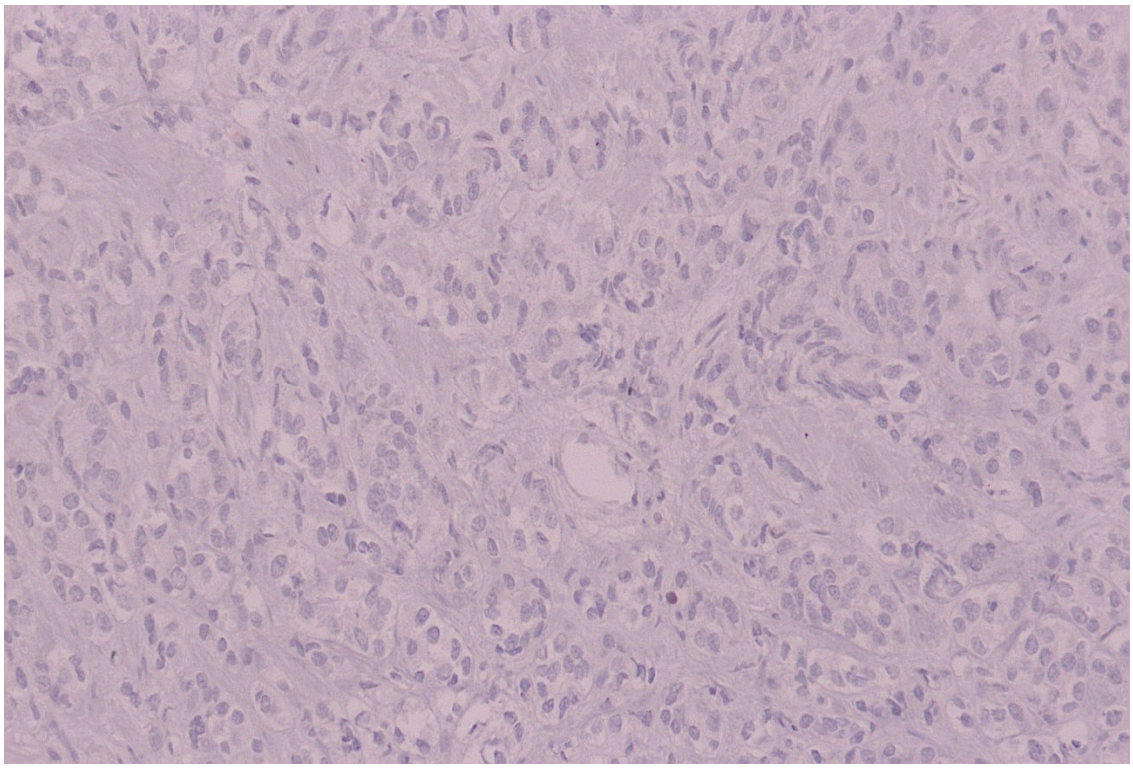
Photomicrograph 5: Group III – The nucleus staining a moderate brown colour. Moderately positive for GATA3 marker (IHC- 20X)



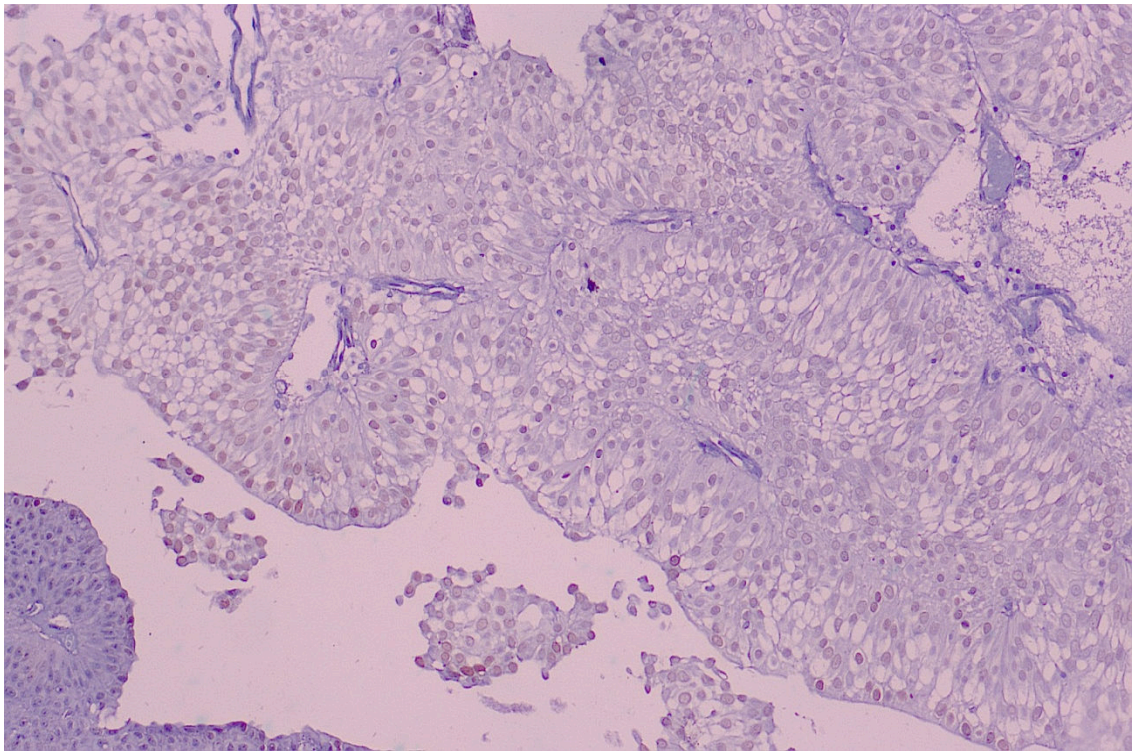
Photomicrograph 6: Group IV – The nucleus staining a dark brown colour. Strongly positive for GATA3 marker (IHC- 40X)



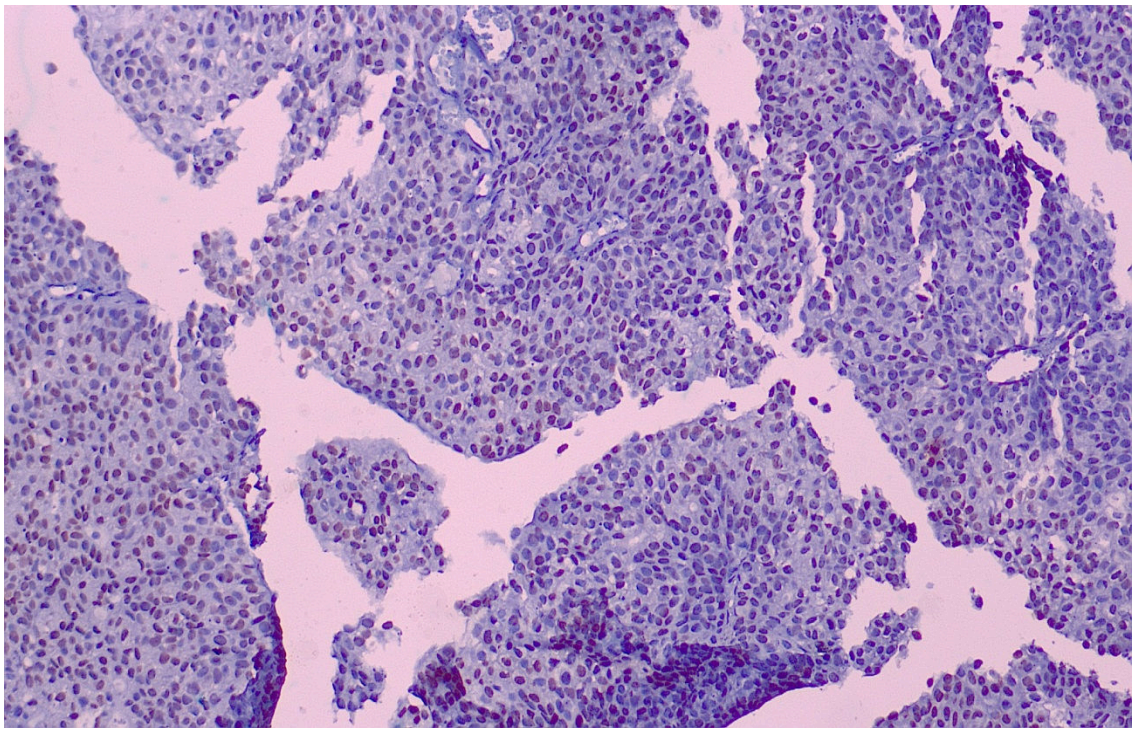
Photomicrograph 7: High Grade Prostate adenocarcinoma (Gleason score= 5+5=10, Group grade 5) (H&E -40X)



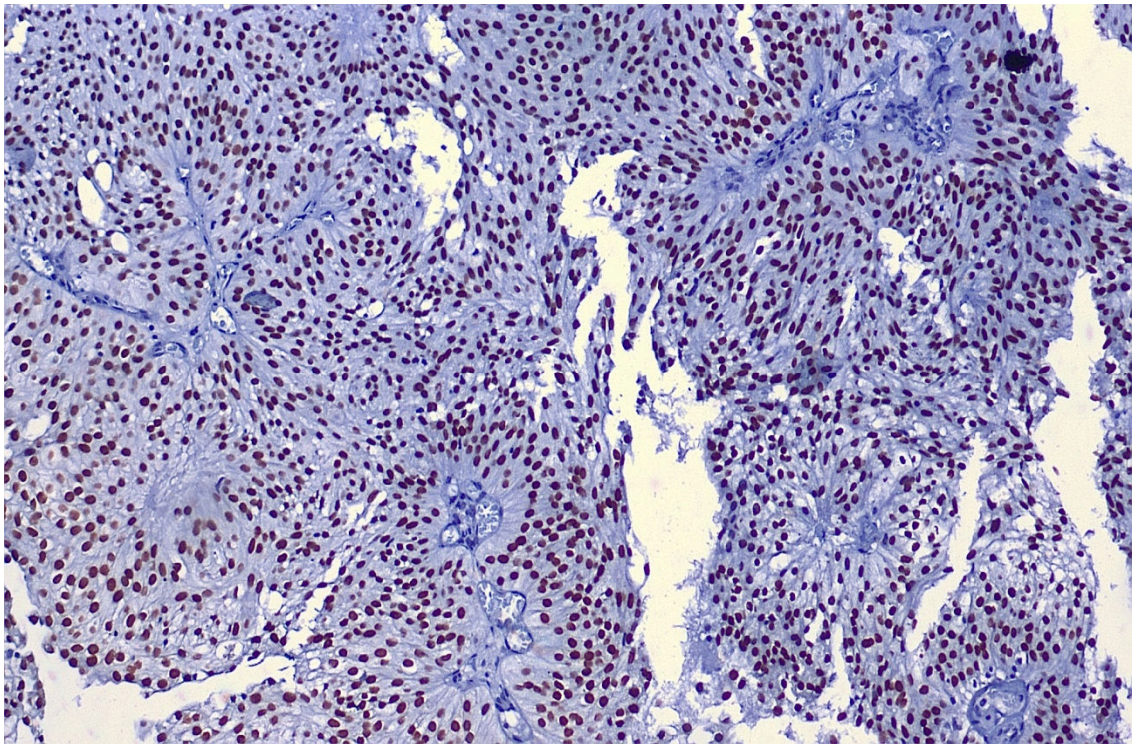
Photomicrograph 8: Prostate adenocarcinoma staining negative for GATA3 marker (IHC-40X)



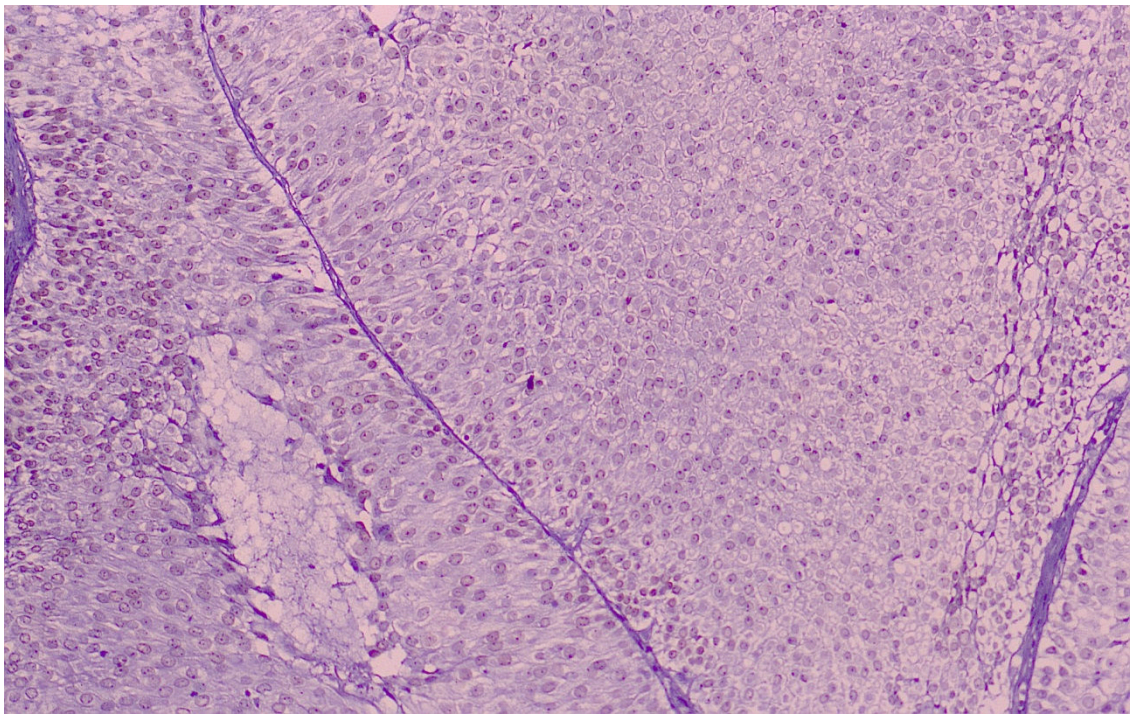
Photomicrograph 9: Urothelial carcinoma positive for GATA3 marker
Staining Intensity – 1
Percentage of tumour cells stained – 3
(IHC- 20X)



Photomicrograph 10: Urothelial carcinoma positive for GATA3 marker
Staining Intensity – 3
Percentage of tumour cells stained – 3
(IHC- 20X)



Photomicrograph 11: Urothelial carcinoma positive for GATA3 marker
Staining Intensity – 3
Percentage of tumour cells stained – 4
(IHC- 20X)



Photomicrograph 12: Urothelial carcinoma positive for GATA3 marker
Staining Intensity – 2
Percentage of tumour cells stained – 2
(IHC- 20X)

ANNEXURE- X

KEY TO MASTER CHART

IHC	–	Immunohistochemistry
TCS	–	Tumour cells stained
SI	–	Staining intensity
IMMUNO SCORE	–	Immunoreactivity Score
INTRPRTN	–	Interpretation
PLEO	–	Pleomorphism
LVI	–	Lymphovascular invasion
PNI	–	Perineural invasion
TIL	–	Tumour infiltrating lymphocytes
cm	–	Centimeters
LGUC	–	Low grade urothelial carcinoma
HGUC	–	High grade urothelial carcinoma
P	–	Positive
N	–	Negative
PR	–	Present
AB	–	Absent
LW	–	Lateral wall
BN	–	Bladder neck
S	–	Smoking
T	–	Tobacco use
OBST	–	Obstructive
PSA	–	Prostate Specific Antigen

SL NO:	IP NO:	AGE	SEX	SAMPLE	GRADE	IHC	TCS(%)	SI	IMUNNO SCORE	INTRPRTN	NUCLEAR PLEO	MITOSIS	NECROSIS	LVI	PNI	TIL	SITE	SYMPTOMS	HABITS	Size (cm)
1	1009159	59	M	1102/20A	LGUC	P	2	3	6	III	1+	< 5	AB	AB	AB	PR	LW	Hematuria	S+T	4.1
2	1016515	87	M	1731/20A	LGUC	P	4	3	12	IV	1+	< 5	AB	AB	AB	PR	LW	Hematuria	NONE	1
3	1004932	65	M	862/20	HGUC	P	2	2	4	II	3+	> 10	AB	PR	AB	PR	LW	Hematuria	S+T	3
4	1003694	79	M	747/20	HGUC	N	0	0	0	I	3+	> 10	PR	AB	AB	PR	OTHERS	Hematuria	NONE	2
5	1013172	57	M	1407/20B	HGUC	P	4	3	12	IV	3+	> 10	PR	PR	AB	PR	OTHERS	PAIN AND Hematuria	NONE	2.5
6	1009805	67	M	1168/20H	HGUC	N	0	0	0	I	3+	> 10	AB	PR	AB	PR	LW	Hematuria	S+T	3.5
7	1008632	66	M	1077/20	HGUC	P	2	2	4	II	3+	> 10	AB	PR	AB	PR	OTHERS	PAIN,Hematuria, INCREASED FREQUENCY	S+T	1.8
8	1012275	30	F	1339/20A	HGUC	P	2	1	2	II	3+	5 to 10	PR	AB	AB	PR	OTHERS	PAIN,Hematuria, INCREASED FREQUENCY	NONE	1.4
9	956025	71	M	2583/19	LGUC	P	4	3	12	IV	1+	<5	AB	AB	AB	PR	LW	PAIN, HEMATURIA, INCREASED FREQUENCY	NONE	3.5
10	981271	75	M	4411/19B	LGUC	P	4	3	12	IV	1+	5 to 10	AB	PR	AB	PR	OTHERS	PAIN,Hematuria, INCREASED FREQUENCY	NONE	3.8
11	965458	57	M	3146/19A	LGUC	P	4	3	12	IV	1+	<5	AB	PR	AB	PR	LW	PAIN	S	1.6
12	963665	43	M	3060/19A	LGUC	P	4	3	12	IV	1+	<5	AB	AB	AB	PR	OTHERS	PAIN, INCREASED FREQUENCY	S	1.7
13	930472	45	F	721/19	LGUC	P	4	3	12	IV	1+	<5	AB	AB	AB	PR	LW	HEMATURIA	NONE	1.8
14	929888	37	M	674/19B	LGUC	P	4	3	12	IV	1+	<5	AB	AB	AB	AB	LW	HEMATURIA, OBST SYMPTOMS	NONE	3
15	945947	66	M	1851/19B	LGUC	P	4	3	12	IV	2+	5 TO 10	AB	AB	AB	AB	LW	HEMATURIA, OBST SYMPTOMS	T	2.5
16	981017	80	M	4099/19C	HGUC	N	0	0	0	I	3+	>10	PR	AB	AB	PR	OTHERS	HEMATURIA	NONE	6.3
17	991237	80	M	4767/19H	HGUC	P	1	2	1	II	3+	>10	PR	PR	PR	PR	LW	HEMATURIA, PAIN	S+T	5
18	955346	81	M	2537/19	HGUC	P	4	3	12	IV	3+	5 to 10	PR	AB	AB	PR	LW	HEMATURIA	T	3
19	963438	55	F	3040/19C	HGUC	P	3	3	9	IV	3+	>10	PR	PR	PR	PR	LW	HEMATURIA, INCREASED FREQUENCY, OBST SYMPTOMS	T	2.5
20	961717	51	M	2994/19B	HGUC	P	4	3	12	IV	3+	5 to 10	AB	AB	AB	PR	LW	HEMATURIA, INCREASED FREQUENCY	S	7
21	923987	75	M	280/19	HGUC	N	0	0	0	I	3+	>10	AB	AB	AB	PR	LW	PAIN, HEMATURIA	S+T	1
22	943020	64	M	1651/19A	HGUC	P	1	2	2	II	3+	>10	AB	PR	AB	PR	LW	PAIN, HEMATURIA, OBST SYMPTOMS	S	2.5
23	943380	60	M	1682/19	HGUC	P	3	3	9	IV	3+	>10	PR	PR	AB	PR	LW	PAIN, HEMATURIA	S	4.8
24	929071	69	M	619/19A	HGUC	P	2	2	4	II	3+	>10	PR	PR	AB	PR	LW	HEMATURIA, INCREASED FREQUENCY	S+T	1.5
25	945405	49	M	1833/19H	HGUC	N	0	0	0	I	3+	>10	AB	PR	AB	PR	OTHERS	PAIN	NONE	5.6
26	867062	62	M	1099/18A	LGUC	P	3	1	3	II	1+	<5	PR	AB	AB	AB	LW	PAIN,HEMATURIA	S	4
27	879644	41	M	1997/18A	LGUC	P	3	2	6	III	2+	>10	PR	AB	AB	AB	LW	HEMATURIA	NONE	2
28	878029	30	M	1910/18	LGUC	N	0	0	0	I	1+	<5	AB	AB	AB	PR	BN	HEMATURIA	T	2
29	909462	40	F	4083/18C	LGUC	N	0	0	0	I	1+	<5	AB	AB	AB	AB	OTHERS	PAIN, HEMATURIA, OBST SYMPTOMS	T	5
30	869759	60	M	1335/18	LGUC	N	0	0	0	I	2+	<5	AB	AB	AB	PR	OTHERS	HEMATURIA	S	2
31	879451	66	M	2001/18A	LGUC	P	3	2	6	III	2+	<5	AB	AB	AB	PR	LW	HEMATURIA	S	1.5
32	904718	60	M	3766/18	HGUC	P	3	3	9	IV	3+	5 TO 10	PR	AB	AB	AB	LW	HEMATURIA	SS	2
33	901698	87	M	3557/18A	HGUC	P	3	3	9	IV	3+	>10	PR	AB	AB	AB	LW	PAIN, HEMATURIA, OBST SYMPTOMS	S	3
34	874156	65	F	1652/18B	HGUC	P	2	2	4	II	3+	>10	PR	PR	PR	PR	LW	PAIN, HEMATURIA, OBST SYMPTOMS	T	3.8
35	884904	47	M	2471/18A	HGUC	N	0	0	0	I	3+	>10	PR	PR	PR	PR	BN	PAIN,Hematuria, INCREASED FREQUENCY	S+T	4
36	883919	84	M	2353/18B	HGUC	P	3	2	6	III	3+	5 TO 10	PR	PR	AB	PR	BN	HEMATURIA	S+T	3
37	895495	70	M	3193/18C	HGUC	P	2	2	4	II	2+	<5	PR	PR	AB	PR	OTHERS	HEMATURIA	S	3
38	849211	58	M	72/18C	HGUC	P	3	3	9	IV	3+	>10	PR	PR	AB	PR	LW	HEMATURIA, OBST SYMPTOMS	T	4
39	911943	64	M	4267/18B	HGUC	N	0	0	0	I	3+	>10	PR	PR	AB	PR	LW	HEMATURIA	S+T	3.2
40	859760	77	M	652/18B	HGUC	P	2	2	4	II	2+	5 TO 10	AB	PR	AB	PR	OTHERS	HEMATURIA	S+T	4.4

SL NO:	IP NO:	AGE	SEX	SAMPLE NO:	TYPE OF CANCER	GLEASON SCORE	GROUP GRADE	PSA(ng/ml)	PNI	TCS(%)	SI	IMMUNO SCORE	INTRPRTN	IHC
1	831151	77	M	324/20F	PROSTATE ADENOCARCINOMA	3+3=6	1	14	POSITIVE	0	0	0	I	NEGATIVE
2	1014075	82	M	1607/20C	PROSTATE ADENOCARCINOMA	4+4=8	4	38	POSITIVE	0	0	0	I	NEGATIVE
3	1014167	79	M	1516/20C	PROSTATE ADENOCARCINOMA	4+5=9	5	56.2	POSITIVE	0	0	0	I	NEGATIVE
4	5582055	77	M	827/20L	PROSTATE ADENOCARCINOMA	3+4=7	2	48.13	POSITIVE	0	0	0	I	NEGATIVE
5	1005127	70	M	826/20B	PROSTATE ADENOCARCINOMA	4+4=8	4	54	NEGATIVE	0	0	0	I	NEGATIVE
6	1003418	70	M	708/20D	PROSTATE ADENOCARCINOMA	3+4=7	2	23.1	NEGATIVE	0	0	0	I	NEGATIVE
7	1017069	73	M	1805/20	PROSTATE ADENOCARCINOMA	4+4=8	4	337	POSITIVE	0	0	0	I	NEGATIVE
8	21133497	70	M	1086/20B	PROSTATE ADENOCARCINOMA	4+3=7	3	47	POSITIVE	0	0	0	I	NEGATIVE
9	5718310	60	M	983/20L	PROSTATE ADENOCARCINOMA	3+4=7	2	13.99	POSITIVE	0	0	0	I	NEGATIVE
10	1012000	65	M	1308/20B	PROSTATE ADENOCARCINOMA	5+5=10	5	51	NEGATIVE	0	0	0	I	NEGATIVE
11	5822924	65	M	2111/20J	PROSTATE ADENOCARCINOMA	4+5=9	5	711	POSITIVE	0	0	0	I	NEGATIVE
12	5718310	54	M	1462/20L	PROSTATE ADENOCARCINOMA	4+5=9	5	100	POSITIVE	0	0	0	I	NEGATIVE
13	3016135	84	M	1301/20	PROSTATE ADENOCARCINOMA	4+4=8	4	36.5	NEGATIVE	0	0	0	I	NEGATIVE
14	3020879	75	M	1280/20B	PROSTATE ADENOCARCINOMA	4+4=8	4	5000	POSITIVE	0	0	0	I	NEGATIVE
15	1014075	82	M	1514/20K	PROSTATE ADENOCARCINOMA	3+3=6	1	39.5	NEGATIVE	0	0	0	I	NEGATIVE
16	1016997	82	M	1804/20K	PROSTATE ADENOCARCINOMA	3+4=7	2	8.23	NEGATIVE	0	0	0	I	NEGATIVE
17	5800255	77	M	820/20I	PROSTATE ADENOCARCINOMA	3+4=7	2	48	POSITIVE	0	0	0	I	NEGATIVE
18	5442995	65	M	3749/19K	PROSTATE ADENOCARCINOMA	4+4=8	4	438	POSITIVE	0	0	0	I	NEGATIVE
19	963951	72	M	3148/19C	PROSTATE ADENOCARCINOMA	3+4=7	2	10.22	NEGATIVE	0	0	0	I	NEGATIVE
20	5361630	71	M	2984/19N	PROSTATE ADENOCARCINOMA	4+3=7	3	150	NEGATIVE	0	0	0	I	NEGATIVE
21	960394	75	M	2869/19N	PROSTATE ADENOCARCINOMA	5+4=9	5	38.9	NEGATIVE	0	0	0	I	NEGATIVE
22	927319	76	M	518/19 B	PROSTATE ADENOCARCINOMA	5+4=9	5	560	NEGATIVE	0	0	0	I	NEGATIVE
23	5204155	66	M	1296/19A	PROSTATE ADENOCARCINOMA	5+4=9	5	87.7	POSITIVE	0	0	0	I	NEGATIVE
24	5204102	63	M	1293/19N	PROSTATE ADENOCARCINOMA	3+4=7	2	11.2	NEGATIVE	0	0	0	I	NEGATIVE
25	990423	77	M	4278/19J	PROSTATE ADENOCARCINOMA	4+3=7	3	34	POSITIVE	0	0	0	I	NEGATIVE
26	989606	85	M	4665/19A	PROSTATE ADENOCARCINOMA	4+4=8	4	36	POSITIVE	0	0	0	I	NEGATIVE
27	987729	76	M	4543/19A	PROSTATE ADENOCARCINOMA	5+4=9	5	419	POSITIVE	0	0	0	I	NEGATIVE
28	976300	75	M	3815/19E	PROSTATE ADENOCARCINOMA	3+4=7	2	189	NEGATIVE	0	0	0	I	NEGATIVE
29	972068	69	M	3701/19B	PROSTATE ADENOCARCINOMA	4+4=8	4	94	NEGATIVE	0	0	0	I	NEGATIVE
30	941464	72	M	1574/19J	PROSTATE ADENOCARCINOMA	4+5=9	9	3049	NEGATIVE	0	0	0	I	NEGATIVE
31	3049405	64	M	2553/19F	PROSTATE ADENOCARCINOMA	4+3=7	3	22.5	POSITIVE	0	0	0	I	NEGATIVE
32	956293	58	M	2596/19B	PROSTATE ADENOCARCINOMA	3+5=8	4	60	POSITIVE	0	0	0	I	NEGATIVE
33	5480470	74	M	4128/19I	PROSTATE ADENOCARCINOMA	4+3=7	3	80	POSITIVE	0	0	0	I	NEGATIVE
34	989412	50	M	4669/19F	PROSTATE ADENOCARCINOMA	4+4=8	4	204	POSITIVE	0	0	0	I	NEGATIVE
35	955980	74	M	2570/19L	PROSTATE ADENOCARCINOMA	4+4=8	4	42.03	POSITIVE	0	0	0	I	NEGATIVE
36	3607345	75	M	1242/19B	PROSTATE ADENOCARCINOMA	5+4=9	5	88.79	POSITIVE	0	0	0	I	NEGATIVE
37	5300017	80	M	2509/19A	PROSTATE ADENOCARCINOMA	4+4=8	4	6.77	POSITIVE	0	0	0	I	NEGATIVE
38	965804	69	M	3198/19L	PROSTATE ADENOCARCINOMA	3+4=7	2	60.21	NEGATIVE	0	0	0	I	NEGATIVE
39	978856	65	M	4728/19L	PROSTATE ADENOCARCINOMA	3+3=6	1	59	NEGATIVE	0	0	0	I	NEGATIVE
40	5571840	73	M	2989/19C	PROSTATE ADENOCARCINOMA	4+3=7	3	150	NEGATIVE	0	0	0	I	NEGATIVE