CYTO-HISTOLOGICAL STUDY OF URINARY BLADDER NEOPLASMS

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CERTIFICATE

This is to certify that this dissertation entitled **"CYTO-HISTOLOGICAL STUDY OF URINARY BLADDER NEOPLASMS"** is a bonifide work done by **Dr. S. SASIKALA,** in partial fulfillment of regulations of the **TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY, Chennai.**

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DECLARATION

I declare that this dissertation entitled "**CYTO-HISTOLOGICAL STUDY OF URINARY BLADDER NEOPLASMS"** has been conducted by me under the guidance and supervision of **Prof.N.SHANTHI VIJAYALAKSHMI, M.D.** in the Institute of Pathology and Electron Microscopy, Madras Medical College, Chennai. It is submitted in partial fulfillment of the requirements for the award of the M.D., pathology, September 2006 examination to be held under Dr.M.G.R.Medical University, Chennai. This has not been submitted by me for the award of any degree or diploma from any other University.

Dr.S.SASIKALA

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CONTENTS

INTRODUCTION

Urinary cytology has an increasingly prominent role in the multidisciplinary diagnostic approach to bladder cancer. It is used as a valuable adjunct to cystoscopy and biopsy for diagnosis and follow up of patients with bladder cancer.

Bladder cancer accounts for 7% of all cancers in male and 2% of all cancers in female. In the year 2002, in Chennai Bladder cancer accounted for 2.28 % of all cancers in male and 0.78 % of all cancers in female[29].

Cytological examination of voided urine is a non-invasive screening test for bladder tumors, which can be carried out in remote areas of the country. By cytology one can even classify type and grade malignancy.

In 1945 Papaniculoau and Marshall recommended cytological examination of urinary sediment for diagnosis and follow up of patients with urological malignancies.

The prognostic value of conventional cytology to monitor patients with superficial bladder carcinoma is well established. While cystoscopy and biopsy are optimum for diagnosis of visible disease, the entire bladder mucosa can be sampled by cytology, enabling detection of occult urothelial abnormalities.

Traditionally cytological examinations have been used to detect in situ and early invasive bladder cancer in high-risk population and in conjunction with cystoscopy and biopsy to diagnose new or recurrent bladder tumor.

Cytology also has been used to identify persistent tumor after transurethral resection.

Cystoscopy remains the standard for the diagnosis and surveillance of bladder tumors, allowing the lesions to be mapped and sampled. However, cystoscopy cannot explore the whole bladder urothelium, and cannot diagnose all carcinoma in situ cases or lesions of upper urinary tract. Thus, it must be combined with urinary cytology, particularly in search for tumor cells from high-grade lesions, wherever their location in the urinary tract.

Urine cytology can detect bladder tumor before it can be detected cystoscopically. Urine cytology is still indispensable in the management of patients with transitional cell carcinoma. It remains as a gold standard for bladder cancer screening.

AIMS

1. To study the value of urinary cytology in the diagnosis of bladder cancer.

2. To correlate urine cytology with biopsies of the bladder neoplasm.

3. To analyze the reason for false positive and false negative cytological results.

4. To correlate urine cytology findings with cystoscopic findings.

REVIEW OF LITERATURE

Cancer bladder was first noted in man in aniline industry in 1895. Cancer bladder is three times more common in men than in women and twice as common among whites as among blacks. The average patient is 65 to 70 years old and most patients are older than 50 years of age.

The bladder temporarily stores concentrated toxic products of renal excretion and is vulnerable to environmental carcinogens. Most important risk factors for the development of transitional cell carcinoma are aniline dyes containing arylamines, e.g.benzidine, beeta naphthylamine. High-risk occupations include chemical, dye, textile, rubber, and plastic workers as well as painters and hairdressers. Cigarette smoking is a risk factor for bladder cancer. Certain drugs [eg.chemotherapeutic agents, phenacetin and opiates] are associated with the development of bladder cancer.

Schistosoma haematobium infection is a risk factor for sqamous cell carcinoma. Previous exposure of the bladder to radiation increases the risk of urothelial carcinoma. Many genetic alterations are observed in urothelial cell carcinoma, comman are chromosome 9 monosomy or deletions of 9_p and 9_q, deletions of 17_p, 13_q, 11_p and 14_q. The pathway, which is initiated by deletions of tumor suppressor genes on 9_p and 9_q leads to superficial papillary tumors, a few of which may then acquire P_{53} mutations and progress to invasion. The second pathway, which is initiated by P_{53} mutations leads to carcinoma in situ and with loss of chromosome 9, progresses to invasion.

The urocarcinogens, by injuring the coding units of the urothelial cells, render them defective, leading to hyperplasia and neoplasia, with the action of promoters. Neoplastic urothelial cells that penetrate the submucosa and/or muscularis are autonomous[27].

TUMORS OF THE URINARY BLADDER[17]

Benign epithelial tumors

Typical papilloma
Inverted papilloma
Villous adenoma
Mucinous cystadenoma of the urachus
Squamous papilloma

Malignant epithelial tumors

Transitional cell carcinoma
Papillary
(1) Non-invasive
(2) Invasive

Non-papillary
(1) Transitional cell carcinoma in-situ
(2) Invasive transitional cell carcinoma

Squamous cell carcinoma.
Adenocarcinoma
Carcinomas of more than one histologic type

Mesenchymal tumors

Benign
Malignanat

(1) Leiomyosarcoma
(2) Rhabdomyosarcoma
(3) Others

Mixed tumors

Adenofibromas and adenosarcomas
Carcinosarcoma

Hematopoietic neoplasms

Lymphoma
Leukemia
Plasmacytoma

Miscellaneous primary tumors

Paraganglioma
Carcinoid
Malignant melanoma
Dermoid cyst
Yolk sac tumor

Metastatic tumors

URINE CYTOLOGY

Tumor cells in the urine were identified first by Sanders in 1864 and Dickson in 1869. In 1892, Ferguson suggested that microscopic examination of the urine sediment, except for cystoscopy was the best method for diagnosing tumors of the bladder. Rovising in 1895 reported malignant cells in the urine sediment of 3 out of 7 patients with kidney tumors.

However, in 1928 Zemansky correlated urinary findings with subsequent tissue examination in 46 cases of suspected tumors and called the

urine unfit for cytologic study. Interest in cytologic diagnosis waned until the studies by Papaniculaou and Marshell in 1945, supplemented by Papniculaou a year later demonstrated the usefulness of smear technique in diagnosing cancers of urinary organs. Because of the normal desquamation from epithelial surfaces, which increases in the presence of neoplasia, they considered the urine sediment most suitable for the identification of cells with malignant characteristics[32].

Since Papanicolaou and Marshall demonstrated in 1945 that a bladder tumor undergoes exfoliation, which renders possible its recognition by detection of abnormal cells in the urine, cytology has become an important adjuvant for the diagnosis and follow up of patients with cancer. In 1964 Umiker reviewed the literature on the value of urinary cytology and found positive results ranging from 26.1 to 100% with an average of 71.6%. In 1972, Harris and associates used the principle of gastric washing for cytology, which was reported previously by Villarded, and performed bladder irrigation, his yield improved by 30% over urinary cytology. In 1970, Esposti and associates emphasized the importance of the pathological grading of the tumor in influencing the cytological results[43].

Tumors of the urinary tract are relatively inaccessible to direct biopsy and the tumors are often multifocal. Since the entire mucosal surface is bathed in urine, in theory urine is the perfect specimen to examine for evidence of tumor in the urinary tract.

Urinary cytology is useful in the detection of

1. Urothelial carcinoma in its preclinical phase.
2. Carcinoma in situ.
3. Malignant change in papilloma.
4. Follow up of patients after treatment to detect any residual or recurrent tumors.

Cytologic examination of the sediment of voided urine is the only noninvasive method of detection, diagnosis and follow up of tumors of the bladder and other anatomic components of the lower urinary tract. Urine cytology is a cost effective method.

Urine cytology remains gold standard for bladder cancer screening. It is the test against which all others are compared when evaluating potential bladder tumor markers. It has excellent specificity with few false positive cases. It detects high grade malignant cells even before a cystoscopically distinguishable gross lesion is present[11]. Urine cytology is capable of detecting malignant non-exophytic tumor types that ultrasound cannot detect[34].

The study conducted by M. N. EL-Bolkainy[30] 1980 showed that the cytology is highly effective in the primary diagnosis of high grade transitional cell carcinoma with a positive rate of 93-94.7%.

The study conducted by Leopold G. Koss et al[24] 1985 also showed the cytology of voided urine is highly reliable in the diagnosis of high grade tumors with a sensitivity of 94.2%. In primary flat carcinoma in situ the sensitivity was 100%.

Deep stage bladder carcinoma exfoliate more cells of a mixed transitional and epidermoid type cells. Superficial stage bladder carcinoma is predominantly associated with a pure population of transitional cell carcinoma cells.

Patients with low-grade non-invasive tumors can be followed up cytologically. Patients with negative cytological findings have a very low risk of recurrence while high-grade cytological abnormalities predict an aggressive tumor course.

Niels Harving et al[33] 1988 in their study, concluded that urinary cytology is also better indicator of the presence of concomitant urothelial atypia than preselected mucosal biopsies.

Post operative (radical operation) examination of urine for malignant cells should be made out to monitor the development of new tumors in the kidney ureter or occasionally in the intestine itself.

Clinical history is imperative for the elimination of misdiagnosis. Pertinent information includes the method of specimen collection, the presence of calculi and past therapies. To use urine cytology most effectively it is important first to understand its advantages and limitations.

Advantages of voided urine cytology.

1. Simple, can be easily collected.
2. Repeated if necessary, with little or no inconvenience for the patient.
3. Inexpensive.
4. Non invasive.

5. Can be done even in remote areas of the country.

6. Detects high grade malignancy before cystoscopy can detect.

7. Samples of urine may contain representative cells from the entire urinary tract. So that the entire system can be surveyed.

Disadvantages of voided urine cytology

1. Low cellularity.
2. Contamination from female genital tract.
3. May contain degenerated cells or reactive transitional cells which may give false positive results.
4. Not localizes the tumor.

An important diagnostic principle is that the higher the grade of the tumor, the more accurate the diagnosis. There are several reasons for diagnostic inaccuracy. Urine is an inhospitable environment for cells. So degenerative changes that make diagnosis difficult are common.

False positive results can occur due to mistaking of atypical cellular changes as malignant. Common causes of cellular atypia are cystitis, senile prostate, calculi, chemotherapy and radiotherapy effects, and polyoma virus infection.

False positive diagnosis of bladder cancer may lead to radical therapy, so a positive urine cytologic diagnosis should always be confirmed histologically before definitive therapy is instituted[12,37]. Most patients with false positive cytological findings may develop a tumor in subsequent years and therefore need repeated examinations of urine for malignant cells.

False negative diagnosis may be of more clinical consequence. Cytologic

diagnosis of papilloma and well differentiated transitional cell carcinoma can be difficult or impossible because the cells are nearly normal appearing. False negative diagnosis may be due to low cellularity, poor preservation or obscuring of malignant cells by inflammatory cells.

Suboptimal Specimen

According to Sheldon Bastacky et al[41] 1999, urinary specimen is considered suboptimal, if the specimen had one or more of the following deficiencies

1. Less than 15 intermediate or basal urothelial cells.
2. Obscuring of malignant cells by red blood cells or inflammatory cells.
3. Poor cellular preservation.

Apparently false positive (false false positive cytology)

Apparently, false positive cytologic results occur when a lesion is actually present but is not confirmed histolologically i.e. histological false negative[37]. It can occur in urine cytology because,

1. Cytologic examination can detect early lesion some times long before they are cystoscopically visible for biopsy[2,6,11,14,32].

2. Sample of urine may contain representative cells from the entire urinary tract.

3. Some epithelial lesions particularly carcinoma in situ tend to slough due to poor cellular cohesion, resulting in exfoliation of abundant tumor cells in the urine and non diagnostic tissue biopsy.

Following newer diagnostic techniques have been studied to increase

diagnostic efficacy.

- Flow cytometry[16,39].
- Image analysis[1].
- Immunology e.g.-blood group isoantigens and monoclonal antibodies to a variety of tumor associated antigens[3,15,16,40,46].
- Immune histochemical analysis of p53 over expression[21].
- Nuclear matrix protein 22 (NMP22) assay[3,15,40,46].
- The aura Tek FDP assay (Fibrin/Fibrinogen Degradation products)[3,40].
- Measuring telomerase activity[3,40].
- Detecting hyaluronic acid/ Hyaluronidase levels[11].

BASIC SPECIMEN

There are three basic types of exfoliated urinary tract specimens:

(1) Voided urine

(2) Catheterized urine, and

(3) Brushing/washing specimens.

"Clean catch" voided urine is recommended for screening purposes. However, in cases in which there is a high clinical suspicion for bladder malignancy, bladder washing may be the method of choice.

Specimens should be processed immediately or refrigerated and processed as soon as possible. If a delay is anticipated, immediate fixation with 50% ethanol may preserve the specimen for several days[16,30,51].

A solution of 25% ethanol [equal volume of 50% ethanol and urine.] is widely used. This percentage is recommended in order to lessen the shrinkage and hardening effects of more concentrated solution[52].

All urine cytology specimens are sufficiently dilute as to require some form of cell concentration. Initially, cytologic findings were assesed on smears made from the sediment of centrifuged specimen. Subsequently developed methods include thin membrane filtration, cytocentrifugation and most recently mono layer technique[11].

Urinary samples can be processed by several techniques including direct smears, membrane filters and cytocentrifugation. All have certain advantages and disadvantages. In general direct smears are easiest to prepare but suffer

from high cell loss and suboptimal display of cellular details. Membrane filters produce the best cytologic details, but are difficult to prepare[51].

Cytyc thinprep liquid based cytology and cytocentrifugation are appropriate methods for cytology based molecular studies but cytocentrifugation remains the quality standard for current treatment of urinary samples because of its lower cost[9].

Processing techniques for urinary samples[25]

Processing technique	**Features**
Direct smear from centrifuged specimen	Easy method, valuable for cytobrush;but shows scant cellularity, degeneration and fragmentation of cells
Cytospin	Good cellularity and preservation of cells;but produces cell clustering suggesting a low-grade papillary tumor, non-cellular contents may obscure the cells, excessive spinning can produce hyperchromasia.
Thin prep	Good cellularity;but expensive, loss of cytoplasmic border and nuclear details
Cellulosic (Millipore) filters	Good cellularity, excellent cytomorphology;but expensive requires fresh samples and experience
Parafin cell block	Valuable in cytobrush samples. Shows excellent tissue architecture;but not recommended for routine urine samples

Papanicolaou stain is usually preferred. Since fine nuclear detail is often crucial to proper diagnosis.

Voided Urine

Normal voided urine usually contains only a few cells. Normal urine

contains approximately 10 cells/ml[51]. Voided urine also contains significant amount of skin and vaginal contamination particularly in female. Increased cellularity may be seen with instrumentation, stones or neoplasms.

The transitional cells in urine have a some what trapezoidal (truncated pyramid or trapezium) shape. The transitional cells in a voided urine specimen are generally relatively uniform. However, the cells can vary from parabasal to relatively large. The nuclei are round to oval with smooth nuclear membranes. The chromatin is delicate or condensed. Small nucleoli may be seen. There is usually a moderate amount of cytoplasm[37].

Urine cytology should be performed on freshly voided urine. A mid morning sample is the most useful. Fractionated cytology did not improve the diagnostic accuracy of urinary cytology and that any part of voided stream is adequate for cytological purposes[22]. Samples should be collected in a clean container and sent to the laboratory immediately. If the specimen has to be transported or kept overnight alcohol should be added to preserve the cells

Cell loss from slides can be minimized by using adhesive fluids. The paraffin blocks of sediments are useful in identification of papillary tumor fragments.

The optimum number of voided urine samples to submit is three. Of the neoplasms that can be diagnosed with voided urine cytology, about 80% will be on the first specimen, 15% on the second, and the rest on the third[24].

Degenerative changes, are common in voided urine specimens. Factors

causing degeneration include high acidity and variable osmolality of the urine. Degeneration of urothelial cells begin prior to exfoliation and continuous while the cells are in contact with urine prior to and after voiding.

Specimen should not be submitted from first morning voiding, 24 hours collection or drainage bags as prolonged exposure of urothelial cells to urine causes degenerative changes.

Catheterized urine

Simple catheterization increases the cellularity over voided urine specimens, and the specimen may be somewhat better preserved. However, it makes the diagnosis of low-grade lesions more difficult because pseudo papillary clusters may be present, and lesions in the urethra may be missed.

Bladder wash

Bladder wash specimens are diagnostically superior to voided urine specimens. The specimens are better preserved, more cellular and there is less contamination of the background than voided urine specimen. It is important that the urologist submit both the saline wash and the urine present in the bladder at cystoscopy ("cysto urine"). Cysto urine does pick up a significant number of additional cases of cancer.

A freshly voided urine specimen should be submitted to detect lesions in the urethra. Although bladder washing is superior to voided urine in diagnosis, it is inconvenient, uncomfortable, relatively expensive, and has a risk of infection.

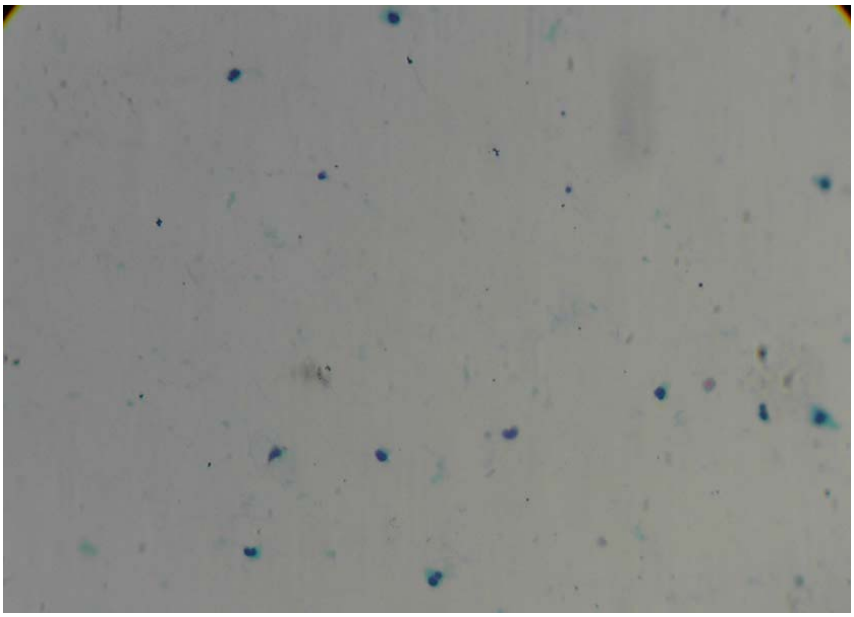
Advantages and Disadvantages of Voided Urine and Bladder Washing

Specimen. [11,16,32,43,45,47,48,49,52]

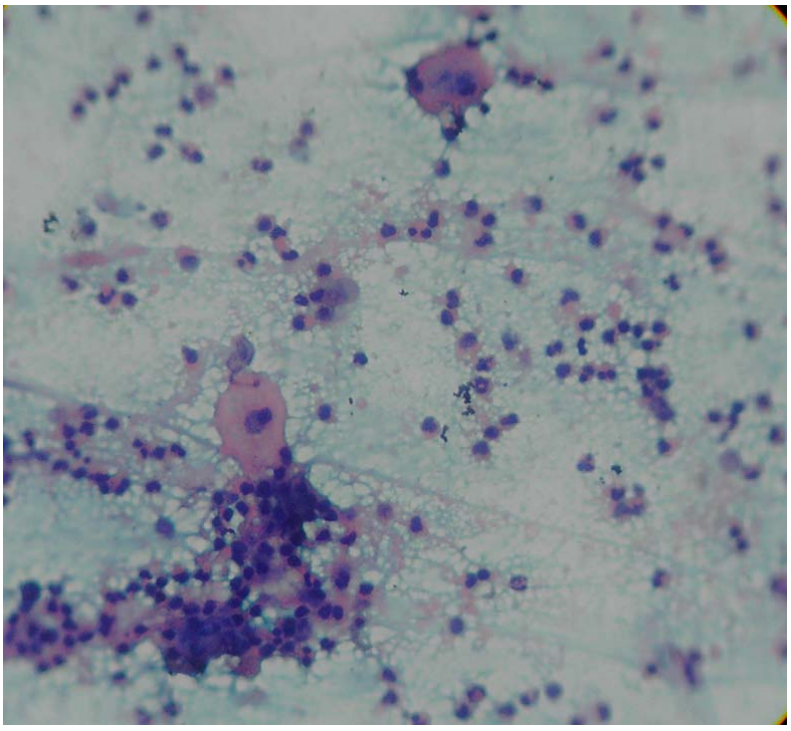
Advantages	Disadvantages
Voided urine	
Easy to obtain specimen	Degeneration
Good sensitivity for high- grade tumor	Few cells, particularly in lowgrade tumor
Sample entire tract	Contamination, especially from female genital tract
Bladder washing	
Excellent preservation	Inconvenient, uncomfortable, expensive
High sensitivity, including low-grade tumors	Possible risk of infection, spread of tumor
Almost no contamination	Limited sample[ie, urethra, upper urinary tract not sampled]

CELLS

Urothelium is composed of superficial layer of large cells which cover several layers of uniform smaller cells like an umbrella, hence named umbrella cells. Superficial cells have abundant cytoplasm and typical concave and convex surfaces with multiple nucleoli. They have a rigid surface membrane which does not round up in fluid environment. So that these elements are readily identified in urinary samples. They can produce and secrete small amount of mucin. Reactive superficial cell may look like a high grade neoplastic cell but truly malignant cells rarely if ever differentiate towards superficial cells. So these cells should not be misinterpreted as neoplastic cells and no cell with a N; C ratio (Nuclear; Cytoplasmic) of 1; 2 or less should be considered malignant.



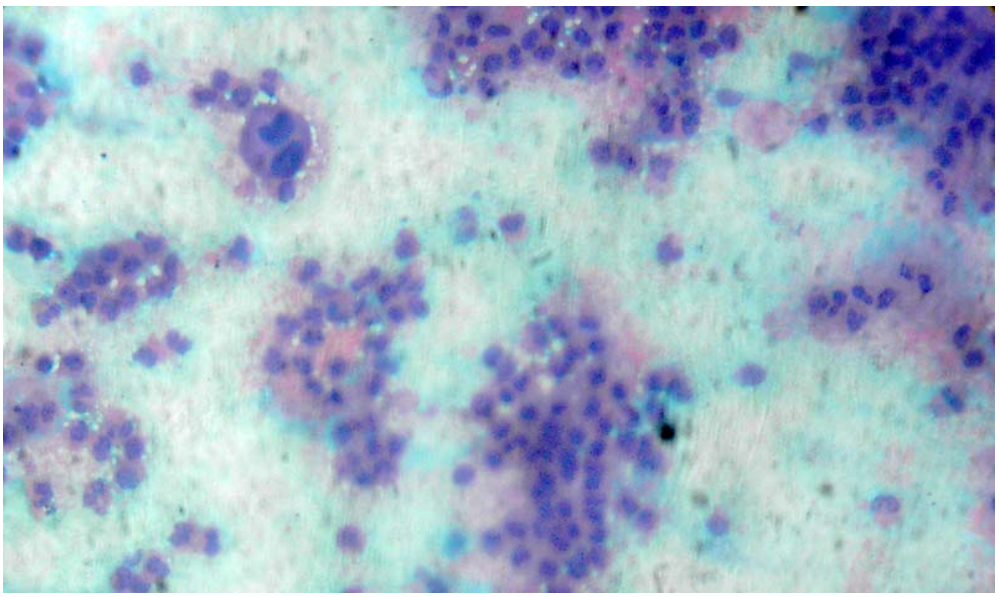
Normal voided urine sediment smear showing subsuperficial transitional cells. Pap stain 400X.



Normal urine sediment smear showing superficial cells and sub superficial small uniform cells. Papstain 400X.

Sub superficial transitional cells lie beneath the superficial cell layer. They are smaller, uniform, normally filled with glycogen which washes out during processing leaving a cleared cytoplasm, surrounding an ovoid nucleus. These cells are usually arranged in 3 to 4 cell thick layers but probably not exceeding 6. They are inactive, replicate every 200 to 500 days. These cells tend to cluster in a urinary specimen. Instrumentation can produce papillary aggregates in urine but these aggregates are having smooth borders and cells are uniform. Papillary configuration perse is an unreliable feature of neoplasia. Reactive sub superficial transitional cells develop cytoplasimic vacuolation, nuclear enlargement and nucleoli become prominent.Most neoplastic cells tend to differentiate toward subsuperficial urothelial cells.It is these elements to which comparison of nuclear characteristics between neoplasia and normal are made[51].

Cytoplasmic vacuolization and prominent nucleoli can be seen in either reactive transitional cells or in high grade transitional cell carcinoma, but their presence excludes low grade transitional cell carcinoma.



Normal urine sediment smear showing superficial cell with double nuclei Pap stain 400X.

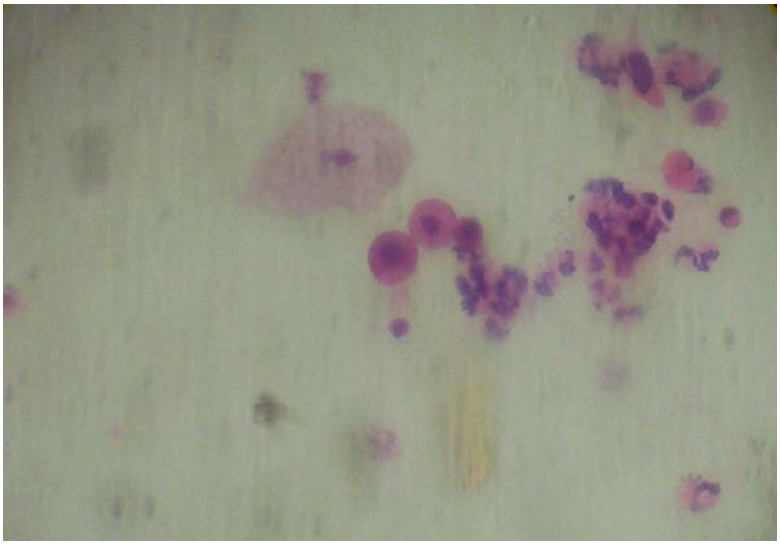
Degenerated Transitional Cells

Degeneration (caused by inflammation, stones, trauma, etc) can result in bizarre transitional cells with darkly condensed coarse or pyknotic chromatin Although such changes can resemble cancer, the chromatin of these benign cells is usually smudged or degenerated. In contrast, the chromatin granules of cancerous cells are characteristically crisp and well preserved. Margination of the chromatin, with central clearing, is also frequent in degenerated transitional cells. Degenerated nuclei may further undergo karyolysis and karyorrhexis and become washed out or broken up. The cytoplasm may disintegrate and be merely a tag attached to the nucleus (comet cell) or absent together[37].

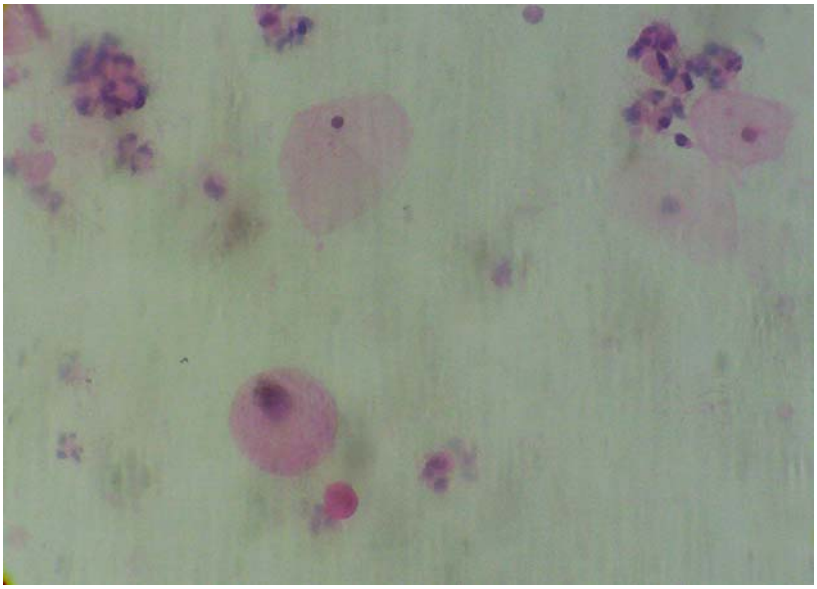
Significance of Papillary Clusters.

Instrumentation, catheterization or even simple manipulation of the bladder produces papillary aggregates, that mimic tissue fragments detached from low grade papillary tumors.

The papillae from transitional cell carcinoma tend to be disorganized and crowded, with irregular borders and mild nuclear atypia, while post instrumentation pseudo-papillae tend to be cohesive, ball-shaped, or papillary clusters with smooth borders outlined by a densely staining cytoplasmic collar. However, when papillary clusters are shed in urine in low grade transitional cell carcinoma, they may be mistaken for artifacts induced by instrumentation[45].



Normal urine sediment smear showing squamous cell and transitional cells. H&E stain 400X.



Normal urine sediment smear showing squamous cell and transitional cells. H&E stain 400X.

Uniform central nuclei, smooth nuclear membranes, fine even chromatin, low N/C ratios, cytoplasmic vacuoles, and general lack of crowding favor a benign diagnosis[16].

Squamous Cells and Squamous Metaplasia

Squamous cells are a common finding in urine specimens. Source of squamous cells are contamination of urine by squamous cells from urethral meatus, female genital tract, cells from trigone, metaplastic squamous cells.

Squamous cells are larger than typical deep transitional cells or about the size of umbrella cells, while the squamous nuclei are smaller and may be pyknotic. Thus, the N/C ratio in a squamous cell is lower than in a transitional cell. The cytoplasm of squamous cells is thin compared with the denser cytoplasm typical of transitional cells. Squamous metaplasia occurs in chronic inflammation or irritation (stones, indwelling catheter). The metaplastic cells are usually mature, intermediate, or superficial squamous cells. Squamous metaplasia should be diagnosed only after exclusion of contamination, thus it requires a catheterized specimen.

Columnar cells

Columnar cells generally account for less than 5% of all cells in a urinary specimen. They commonly appear after prostatic surgery or instrumentation[25].

Red blood cells and inflammatory cells

Normally the urine is virtually free of inflammatory cells and RBCs. Significant numbers indicate disease or trauma and when abundant may

obscure the presence of tumor cells. Hematuria results from any disease or trauma to the Kidney and/or urinary tract and is seen with calculi, neoplasm, infections.

Eight Red Blood Cells per high power field during urine analysis represents abnormal microscopic hematuria[21].

Other cells that may be seen in urine are

1. Renal tubular cells are seen in kidney disease
2. Prostatic cells are seen after prostatic massage
3. Seminal vesicle cells are seen rarely
4. Endometrial cells are seen mainly in case of contamination
5. Lymphocytes are seen in case of cystitis or malignant lymphoma
6. Plasma cells are seen in case of chronic inflammation or multiple myeloma
7. Histiocytes are seen in case of chronic inflammation, radiation or BCG therapy
8. Multinucleated giant cells may be umbrella cells or they can be seen with radiation, BCG therapy or viral infection
9. Eosinophils are seen in case of urinary tract infection, bladder injury or sometimes with bladder cancer

Miscellaneous

1. Corpora amylacea of prostatic origin.
2. Globular or Hyaline, inclusion bodies commonly seen in urinary tract cells. They are associated with degeneration.
3. Sperm, crystals, casts & various organisms can all be seen in urinary

specimen.

Cellular Reaction to Therapeutic Agents

1.Cyclophosphamide is alkylating agent given systemically and metabolized to active form. The active form is concentrated in urine and remains in contact with urothelial cells for relatively prolonged periods until voided. Cyclophosphamide causes cellular atypia that can closely mimic cancer. The presence of multinucleated cells with signs of nuclear degeneration (karyorrhexis, lysis, and nuclear vacuolization) suggests chemotherapy effect.

2. Mitomycin C and Thiotepa are topically applied alkylated agents administrated in their active form for prevention or treatment of bladder neoplasm. The drugs remain in contact with urothelium for limited periods prior to voiding. Their cytologic manifestations are confined almost exclusively to superficial cells, where they cause enlargement of both cytoplasm and nuclei resulting in characteristic but bizarre cells. Nuclei are usually not hyperchromatic but those that are usually have smudgy appearing chromatin. Multiple small nucleoli are common. The cytoplasm is degenerated, vacuolated and frayed.

3. Bacillus Calmette-Guérin [BCG] vaccine is an increasingly common therapeutic agent for bladder cancer that is particularly effective in treating carcinoma in situ. BCG therapy can also cause epithelial atypia, including slight nuclear hyperchromasia with cytoplasmic basophilia[23]. In contrast with cancer, the N/C ratio is preserved and the nuclear membranes are smooth. Significant nuclear pleomorphism, prominent nucleoli, or cytomegaly are not

seen in the transitional cells.

Radiation

Radiation can cause certain cytologic changes, especially multinucleation, cytoplasmic vacuolization and nuclear pyknosis. Bizarre nuclear abnormalities can occur[51].

It is not possible to distinguish with any degree of certainity malignant from non-malignant irradiated urothelial cells in urine. History of previous irradiation should be given to the cytologist.If numerous bizarre cells appear long after radiation, or if there has been an interim period when cytologic appearances were normal, then recurrence is strongly suggested[36].

BLADDER CANCER

Transitional cell carcinoma or urothelial carcinoma accounts for 90% of all primary tumors of bladder.

Most bladder cancers appear as a focal or multifocal expression of widespread abnormality of urothelium, progression of which leads to multicentricity in space and recurrence in time.

Clinical Presentation

Eighty percent of the patients with bladder carcinoma present with gross or microscopic, painless and intermittent hematuria. Twenty percent of patients will complain of vesical irritative symptoms including urinary frequency and urgency. It is important to consider CIS [Carcinoma In Situ.] in any patient

with a history of irritative voiding symptoms. Patients with invasive bladder cancer may have abdominal tenderness or bladder mass or induration.

Biologic Pathways

Two distinct pathways are suggested in urothelial neoplasia. A lowgrade pathway hypothesized in approximately 70% the of cases. It is characterized by progression from hyperplasia/dysplasia to papilloma to low grade transitional cell carcinoma. The lesions have bland cytology and normal blood group isoantigens and are predominantly diploid. Recurrences are frequent and multiple, but only minority of the cases (10 to 15%) progress to muscle invasion.

In the high grade pathway most invasive lesions arise without a history of papillary neoplasms. They progress directly from flat lesions of severe dysplasia/carcinoma in situ to invasive high grade transitional cell carcinoma and are associated with mortality. Such lesions have pleomorphic cytology, manifest high mitotic rates, lack normal blood group isoantigens, and are usually aneuploid[11,50].

The flat carcinoma in situ may precede invasive carcinoma by months or years. A primary goal of urine cytology is to recognize these early flat lesions before they invade, as well as to detect the 10% of papillary lesions that are destined to invade[11].

Cytologic classification of urothelial malignancy.

The lesion acts like it looks[51].

Cellular features of urothelial neoplasia[50,51]

	Low grade	**High grade**
Cells		
Arrangements	Papillary and loose clusters	Isolated and loose clusters
Size	Increased, uniform	Increased, pleomorphic
Number	Often numerous	Variable
Cytoplasm	Homogeneous	Variable May have vacuoles
Nuclear Cytoplasmic ratio	Increased	Increased
	Low grade	**High grade**
Nuclei		
Position	Extremely eccentric	Eccentric
Size	Enlarged	Variable
Morphology	Variable within aggregates	Variable
Borders	Irregular	Irregular
Chromatin	Fine, even	coarse, uneven
Nucleoli	Small/Absent	Variable

General Principles of Cytologic Diagnosis

Higher the grade and the more extensive the tumor, greater the ability to make a cytologic diagnosis. Cells shed from transitional cell carcinoma in situ have a similar cytomorphology as high-grade invasive lesion. One cannot reliably determine whether a lesion is in situ or invasive cytologically.

Differential diagnosis.

Differential Diagnosis includes atypia due to catheterization, cystitis, stones, radiation and chemotherapy.

Differentiating features of reactive cell, low grade and high grade TCC [20,44,50,51]

Feature	Reactive	Low grade TCC	High grade TCC
Groups	Pseudopapillae	Pappillae, loose or crowded clusters	Loose clusters/syncitia/single
Cells	Enlarged, pleomorphic variable in number.	Enlarged, relatively uniform, often numerous.	Enlarged, pleomorphic.
N/C ratio	Normal/increased	Increased(slight to moderate)	Increased(moderate to marked)
Nucleus	Central	Eccentric, enlarged.	Eccentric, pleomorphic
Nuclear membrane	Smooth,thick.	Slightly irregular, thin	Moderate to markedly irregular.
Chromatin	Fine, even	Granular, even	Coarse, dark, irregular
Nucleoli	Often large	Small to none	Macronucleoli in many cells
Cytoplasm	Vacuolated	Homogenous	Often vacuolated also squamous
Background	Inflamed or clean	Clean	Diathesis

High nuclear cytoplasmic ratio, irregular nuclear membrane, nonvacuolated cytoplasm are three key features in the diagnosis of low grade transitional cell carcinoma[42].

Dysplasia

Dysplasia describes a flat, non-invasive premalignant lesion distinguished from reactive or reparative epithelium. The cytologic diagnosis of urothelial

dysplasia is a point of contention. The cells are reported to have a lesser degree of cytologic atypia than transitional cell carcinoma in situ but are similar in appearance to cells from low-grade papillary neoplasms. The chief differences are the small number of dysplastic cells present in urinary specimens, the aggregation of cells into small but loose clusters, a lower N/C ratio [Nuclear Cytoplasmic ratio] and fine evenly distributed chromatin. In truth urothelial dysplasia cannot reliably be segregated from low-grade TCC with subjective criteria of cytology alone.

Coy cells

These single small cells in the background have very high N/C ratio and abnormal slightly hyperchromatic nuclei with slightly coarse chromatin and irregular nuclear membranes. These are the diagnostic cells to look for in low grade transitional cell carcinoma[11].

Papilloma

Papillomas are well-differentiated papillary transitional cell neoplasms. Many papillomas are composed of cells with normal or nearly normal morphologic appearance, making cytologic diagnosis difficult or impossible. Others have morphologic feature of low-grade neoplasm. Therefore only $^{1}/_{3}^{rd}$ to $^{2}/_{3}^{rd}$ of these lesions can be diagnosed cytollogically and the higher diagnostic yield (fewer false negatives) comes at the expense of more false-positive diagnosis[50].

Numerous cells may be exfoliated. Loose clusters and papillary

aggregates commonly occur, but must be differentiated from pseudopapillary clusters related to trauma instrumentation, stones, etc. True papillary fronds with fibrovascular cores are diagnostic of papillary neoplasia[10]. However, their presence is not essential for cytologic diagnosis. The cells and their nuclei are larger than normal deep transitional cells (but not superficial cells). The cell borders are often indistinct. The cytoplasm is homogeneous rather than vacuolated. The nuclei are eccentric and have slightly irregular membranes in the form of notches or creases. The chromatin is fine and evenly distributed. Nucleoli, if present, are small[50,51].

Carcinoma in Situ:

A lesion in which transitional epithelium, of variable thickness, is composed of variably sized abnormal cells with significant nuclear and cytoplasmic abnormalities, limited to the epithelium and never crosses the basal layer.

Squamous Cell Carcinoma

Squamous cell carcinoma can be accurately identified from cells in urine sample. They are usually well differentiated and exfoliate fairly mature cells with an elongated, spindled or fusiform configuration. Cytoplasmic keratinization can often be distinguished. Nuclear border is irregular. Nuclear deformation is common.

Adenocarcinoma

Primary adenocarcinomas are rare in the bladder. The adenocarcinoma

cells tend to cluster and have poorly stained, lucent, vacuolated cytoplasm. Chromatin tends to aggregate along smooth nuclear borders and manifests. Nucleoli are prominent.

Cystoscopy

Cystoscopy and cytology are complementary studies. The low-grade papillary lesions that may be difficult to diagnose cytologically are usually easy to diagnose cystoscopically. The papillae are almost literally waving at the observer through the cystoscope; however, lesions in the dome of the bladder or upper urinary tract may not be visualized[21,37]. Conversely, the high-grade flat carcinoma in situ or early invasive carcinoma, which is much more ominous, is easy to detect cytologically but may be missed cystoscopically even when extensive because it can be mistaken for hyperemia, cystitis, or even normal mucosa[16,38]. Furthermore cystoscopy is a burden for the patient and is not completely free of complication.

Diagnostic correlation between cytologic and cystoscopic examination[37].

Cytology	Cystoscopy	Likely diagnosis
(-)	(-)	No tumor.
(-)	(+)	Well differentiated papillary neoplasm.
(+)	(-)	Carcinoma in situ (or) upper tract tumor.
(+)	(+)	High grade, invasive TCC

Transabdominal ultrasonography has also been used to complement urine cytology in the detection of bladder tumors.

Pitfalls in urinary cytology

- Over interpreting cells with low N/C ratio [Nuclear Cytoplasmic ratio] as Cancer cells.
- Mistaking papillary aggregates as a reliable sign of low grade neoplasia
- Confusing the reactive/regenerative/reparative cell associated with urinary stones with neoplastic cells.
- Over interpreting samples from the upper collecting system.
- Misinterpreting cells in ileal conduits and
- Misunderstanding the cytologic effects of drugs and X ray therapy.

Morphologic features

TCC [Transitional Cell Carcinoma] can arise any where in the bladder.

Common locations are:

Lateral walls 37%
Posterior wall 18%
Trigone 12%
Neck 11%
Ureteric orifices 10%
Dome 8% and
Anterior wall 4%

Pattern of growth may be exophytic or endophytic or a combination of both. Exophytic tumor may adopt a papillary configuration (with central fibrovascular core) or a solid appearance(nodular).

Exophytic tumor results in clusters of tumor cells in the lamina propia

which may be under diagnosed as Von Brunn's nests or cystitis glandularis or cystica. This is referred to as the nested variant of transitional cell carcinoma.

Stromal invasion by transitional cell carcinoma proceeds in two stages. Invasion of the lamina propria and invasion of muscle layer. Muscle invasion is of great consequence because of its influence on therapy and prognosis. Care should be exercised not to misinterpret the inconsistent but sometimes prominent fascicles of muscularis mucosa as belong to the muscularis propria.It is also important not to misinterpret the mature adipose tissue commonly present in the lamina propria or muscularis propria as perivascular soft tissue, in order to avoid a tumor adjacent to fat in a biopsy specimen being badly overstaged.

The cyto architectural variations that may occur in transitional cell carcinomia are

1. Foci of glandular metaplasia are common, usually in the form of intra-cytoplasmic mucin containing vacuoles.
2. Foci of squamous differentiation especially in high grade tumors may be seen.
3. Clear cells may be prominent in transitional cell carcinomia and simulate adenocarcinoma.
4. Micropapillary pattern may occur which resembles that of ovarian serous papillary carcinoma.
5. Rare variant of TCC is characterized by a plasmacytoid appearance that

mimics myeloma.

Bladder Biopsy

Transurethral Resection

Before tumors are resected, their location, number and size should be noted. Endoscopically visualised characterization with predictive value for disease progression include shape (papillary versus sessile and flat), size (less than 2 cm versus greater than 5 cm) and the presence of associated mucosal abnormalities.

After careful inspection has been completed, multiple cold cup biopsies are done. A typical biopsy specimen will provide the pathologist with an adequate sample of tumor, lamina propria and occasionally superficial muscle. The advantages of the cold cup biopsy is that the cellular architecture is undisturbed by cautery artifact. Biopsies are routinely obtained from the tumor, adjacent to the tumor, lateral to each ureteral orifice, the mid bladder base and the prostatic urethra. Biopsies should also be taken from areas adjacent to the tumor and from endoscopically normal sites, as such specimens have predictive value and therapeutic implication in the management of patients with bladder cancer. Cold cup biopsies of mossy granular areas of bladder mucosa may be more important than those of the flat red lesions.

Kenneth B. Cummings et al[21] suggested hot loop resection of the tumor, after adequate biopsy mapping of bladder mucosa. He also insisted that the resection must include sufficient muscle to allow adequate pathologic staging. As an adjunct to hot loop resection, a cold up biopsy of the tumor edge or base

can provide muscle for pathologic staging while reducing charring artifact and the risk of bladder perforation. Video cystoscopy, combined with continuous-flow resectoscope, will allow safe resection in almost all cases.

Radical cystectomy

Radical cystectomy is done for muscle invasive cancer. From radical cystectomy specimen bits should be taken from,

1. Tumor- at least three sections through bladder wall.
2. Bladder neck- one section.
3. Trigone- two sections.
4. Anterior wall- two sections.
5. Posterior wall- two sections.
6. Dome- two sections.
7. Any abnormal looking area in bladder mucosa if not included in previous sections.
8. Ureteral orifices, include intramural portion
9. Ureteral proximal margins
10. In males;sections from prostate and seminal vesicles
11. Other organs present
12. Perivesical lymph nodes if any.

Grading and Staging

***Grading of urothelial tumors*.**[18]

WHO/ISUP Grades (World Health Organization/International Society of Urological Pathology). Adopted as WHO system in 2004.

Urothelial papilloma

Urothelial neoplasm of low malignant potential

Papillary Urothelilal Carcinoma, low grade

Papillary Urothelial Carcinoma, high grade.

WHO Grades (1973)

Urothelial Papilloma

Urothelial neoplasm of low malignant potential

Papillary Urothelial Carcinoma, Grade 1

Papillary Urothelial Carcinoma, Grade 2

Papillary Urothelial Carcinoma, Grade 3

Histologic feature used to classify Urothelial Papillary lesions according to the scheme proposed by the WHO/ISUP [The World Health Organization / International Society of Urological Pathology].[19]

	Papilloma	**Papillary neoplasm of low malignant potential**	**Low grade Papillary Carcinoma**	**High grade Papillary Carcinoma**
Architecture				
Papillae	Delicate	Delicate, Occasionally 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
Mitosos	Absent	Rare, basal	Occasional, at any level	Usually frequent at any level
Umbrella cells	Uniformly present	Present	Usually present	May be absent

***** *If present, small and regular and not accompanied by other features of high grade carcinoma.*

Pathologic T (Primary tumor) staging of bladder carcinoma[18].

AJCC/UICC	Depth of invasion
Non invasive, Papillary	Ta
Carcinoma in situ(Non invasive, flat)	Tis
Lamina Propria invasion	T1
Muscularis Propria invasion	T2
Microscopic extravesicle invasion	T3a
Grass apparent extravesicle invasion	T3b
Invades adjacent structures	T4

[AJCC/UICC, American Joint Commission on Cancer/Union International Contre le Cancer.]

Local spread and metastasis

Bladder carcinom may extend into the ureters, neck of the bladder, urethra, prostatic ducts and seminal vesicle.

Lymphnode metastasis in the pelvic chains are found in 25% of invasive tumors. The most common sites of distant metastatis are the lungs, liver, bone and central nervous system.

Histochemical and Immunohistochemical features.

The co-ordinate expression of CK7 and CK20 is a feature of transitional cell carcinoma.CK20 positivity is common and strong in the high grade tumors. Thrombomodulin and Uroplakin III are two new useful markers for transitional cell carcinomia, but the former is not very specific and the later is only moderately sensitive.

Other markers commonly expressed by these tumors are CEA and cathepsin B (high grade tumors), CA19-9, CD15 (leu M1), survivin and androgen

receptors. Deletion of ABO blood group antigen is a common finding in transitional cell carcinomas particularly in high grade tumors.

Staining for the basal lamina component laminin has been advocated for the detection of early stromal invasion. Tenascin an extra-cellular matrix protein is strongly expressed in invasive, high-grade carcinomas with abundant stroma and is thought to reflect both the severity of the inflammatory infiltrate and the extent of stromal remodeling.

Treatment

Treatment depends on the grade, stage and whether the lesion is flat or papillary. For small localized papillary tumor of low grade, transurethral resection, followed with periodic cystoscopies and urine cytology for rest of their life for tumor recurrence. For multifocal disease in the immediate post operative period, topical chemotherapy is instilled in the bladder. It reduces recurrence. For patients who are at high risk of recurrence and/or progression, topical immunotherapy with BCG is given after the biopsy site is healed.

Radical cystectomy is done for:

1. Tumors invading the muscularis propria,

2. CIS [Carcinoma In Situ] or high grade papillary cancer refractory to BCG [Bacillus Calmette - Guerin] and

3. CIS extending into the prostatic urethra and extending down the prostatic ducts beyond the reach of BCG.

Advanced bladder cancer is treated by chemotherapy.

Prognosis

The prognosis depends on histological tumor type and grade, the depth of invasion, presence or absence of lymphatic or blood vessel invasion and spread to distant sites. The grade and stage of the tumor are important prognostic factors. As the transitional cell carcinoma is a multi focal disease, the status of the residual epithelium is critically important in prognostic assessment of urothelial tumors.

MATERIALS AND METHODS

The present study was undertaken in the Institute of Pathology and Electron Microscopy, Madras Medical College and the Department of Urology, Govt. General Hospital, Chennai.

Voided urine specimens were collected from 54 patients and subsequently bladder biopsy specimens were obtained from these patients and results were correlated.

From August 2004 to Feb 2006 voided urine specimens were obtained from 52 men 2 women patients, who had symptoms suggestive of bladder tumor or were being followed after treatment of transitional cell carcinoma of the bladder. They were in the age group of 28 years-85years.

For cytological examination freshly voided urine was collected in clean container avoiding early morning urine. In case of female patients care was taken to avoid vaginal contamination. Urine examination was repeated thrice. For the purposes of this study, the higher degree of abnormality observed in the three specimens was recorded as the conclusive diagnosis.

Urine specimen was centrifuged 2500 rpm for 20 minutes. The supernatant was poured into disinfectant. The cells in 'cellbutton' mixed with the remaining one or two drops of supernatant, remaining in the side of the tube by gently flicking the tube with a finger.

The resulting cell suspension was removed using a pipette. A single small drop was placed towards one end of a clean, labeled glass slide. It was spread

rapidly and the smears are fixed in 95% Isopropyl alcohol immediately.

The slides were fixed at least for a minimum of 20 minutes. Fixed smears were stained by using H & E stain and papanicoloau stain.

After staining the slides were mounted using DPX [Distrene Dibutylphthalate Xlol].

For Histopathology

The material was obtained from TURBT Specimen, Cystoscopic biopsy specimen and Radical cystectomy specimen.

The samples received were fixed in 10% formation of quantity 20 times, the volume of the specimen. Then processed in automated tissue processor with a conventional timing. Then slides were stained using H &E [Haematoxylin and Eosin] stain.

Papanicoloau staining [5]

Staining Technique

1. Fix smears (while still moist) in equal parts of alcohol and ether for 15 - 30 minutes.
2. Rinse smears in distilled water.
3. Stain in Harri's Haematoxylin for 4 mts.
4. Wash in tap water for1-2mts.
5. Differentiate in acid alcohol.
6. Blue in tap water or 1.5%sodium bicarbonate
7. Rinse in distilled water.
8. Transfer to70% then 95% alcohol for few seconds.

9. Stain in OG-6 for 1-2 mts
10. Rinse in 3 changes of 95% alcohol for few seconds each.
11. Stain in EA-36 for 1 - 2 mts
12. Rinse in 3 changes of 95% alcohol for few seconds each
13. Dehydrate in absolute alcohol, clear in xyol and mount in DPX.[Distrene Dibutylphthalate Xlol]

Result

Nuclei - Blue

Acidophilic cells - Red to Orange

Barophilic cells - Green to blue green

Cells or fragments of tissue penetrated by blood- Orange to Orange Green.

H & E staining [Heamatoxylin and Eosin] for tissue sections[26]

Staining technique

1. Dewax sections, hydrate, through graded alcohol to water
2. Remove fixation pigments if necessary.
3. Stain in alum hematoxylin of choice for a suitable time.
4. Wash well in running tap water until sections 'blue' for 5 minutes or less.
5. Differentiate in 1% acid alcohol (1% Hcl in 70% alcohol) for 5- 10 sec
6. Wash well in tap water until sections are again blue (10 - 15 minutes) or
7. Blue by dipping in an alkaline soluition (eg ammonia water) followed by a 5 min tap water wash.
8. Stain in 1% eosin for 10 minutes
9. Wash in running tap water for 1 - 5 minutes

10. Dehydrate through alcohols, clear and mount.

Results

Nuclei - blue/black

Cytoplasm - varying shades of pink

Muscle fibres - Deep pink/red

RBC[Red blood Cells] -Orange/red

Fibrin - deep pink

For heamatoxylin and eosin staining of cytology smears 0.5% acid alcohol is used

for differentiation

Urine cytology smears were diagnosed as

Negative for malignancy

Atypical/reactive cells

Degenerated cells

Suspicious for malignancy.

Positive for Malignancy

Low grade

High grade

For this purpose of study cytological negative, atypical/ reactive and degenerated smears are combined into one group i.e. cytologic negative. Suspicious and positive smears were combined into another group i.e. cytologic positive.

Histopathology slides were diagnosed as

No malignancy

Low grade transitional cell carcinoma

High grade transitional cell carcinoma

Then Cyto - Histological correlation was done.

Correlation was also done with cystoscopic findings and cytological, histopathalagical findings. Histological diagnosis was based on WHO grading

The tumors showing delicate orderly arranged papillary structures with minimal crowding, with enlarged cells, round to oval slightly variable sized nuclei, inconspicuous nucleoli and occasionally found mitosis were classified as low grade transitional cell carcinoma

The tumors showing disorderly arranged papillary structures with loss of polarity, enlarged cells with pleomorphic nuclei showing multiple prominent nucleoli and frequent mitosis were diagnosed as high grade transitional cell carcinoma.

Criteria for cytological diagnosis

Atypical/reactive cells

Enlarged cells with vacuolated cytoplasm and large centrally placed nuclei with smooth nuclear borders and multiple prominent nucleoli and fine even chromatin were diagnosed as atypical/reactive cells[50,51]

Degenerated cells

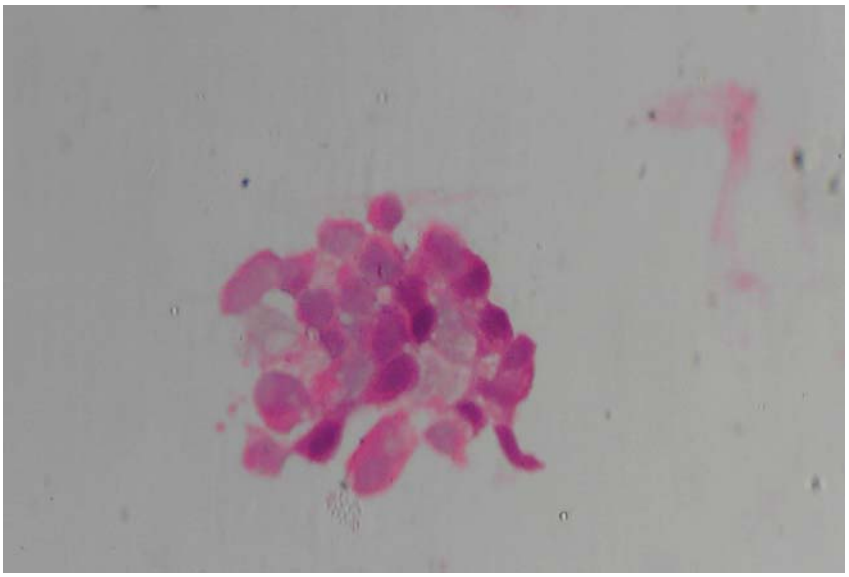
Cells with disintegrated cytoplasm, smudged or degenerated chromatin and degenerated nuclei were diagnosed as degenerated cells[37].

Suspicious cells

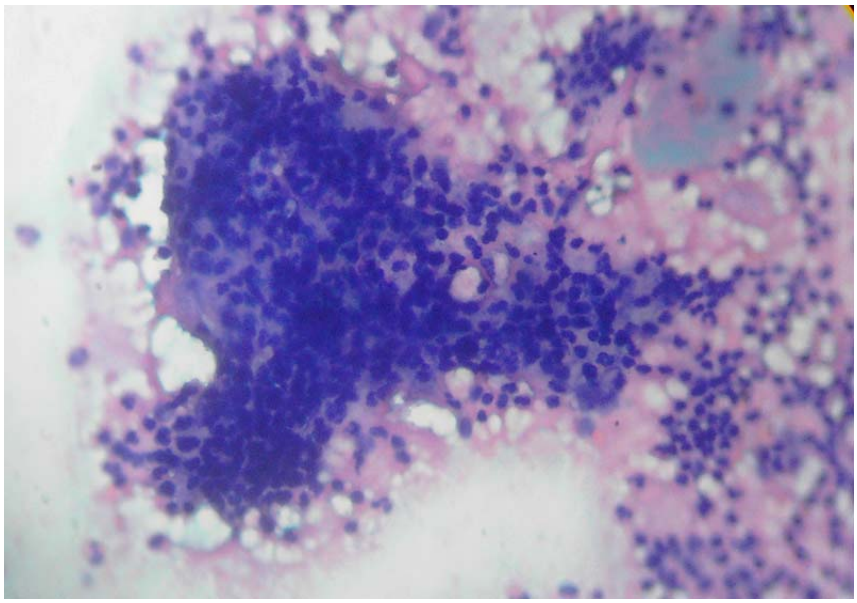
The diagnosis of suspicious was rendered when the evidence was judged to be strongly suggestive of cancer but insufficient for an outright- diagnosis[24].

Low-grade transitional cell carcinoma

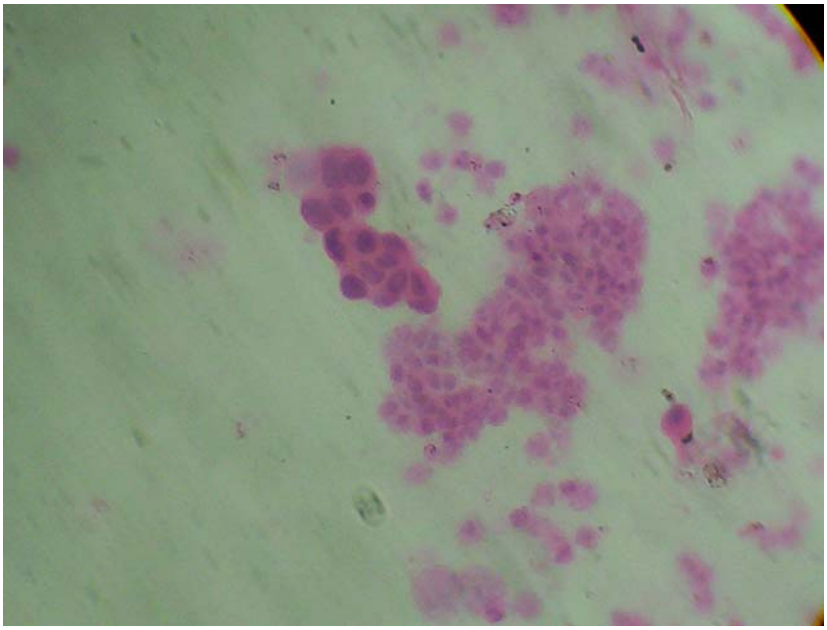
Papillary or loose clusters of large cells with homogeneous cytoplasm and eccentricity placed enlarged nuclei with irregular nuclear borders, fine even chromatin were diagnosed as low grade transitional cell carcinoma[50,51].



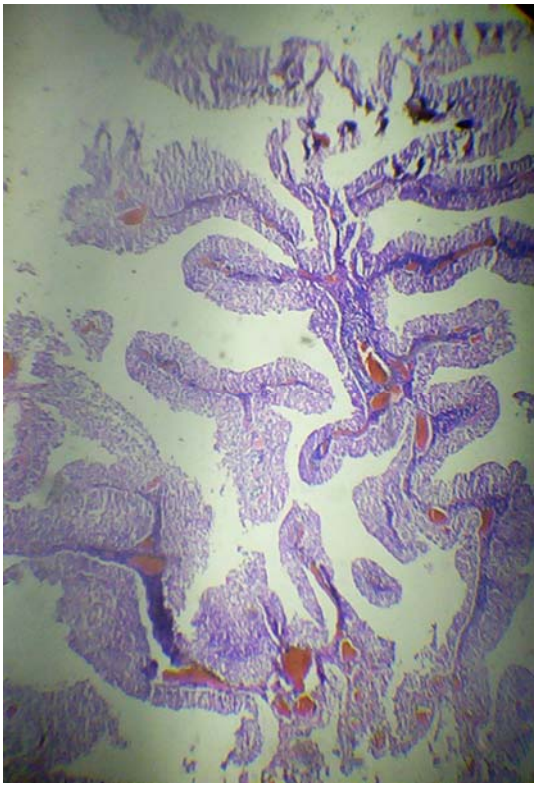
Urine sediment smear showing degenerated cell groups showing vacuolated cytoplasm and degenerated nuclei. H&E stain 400X.



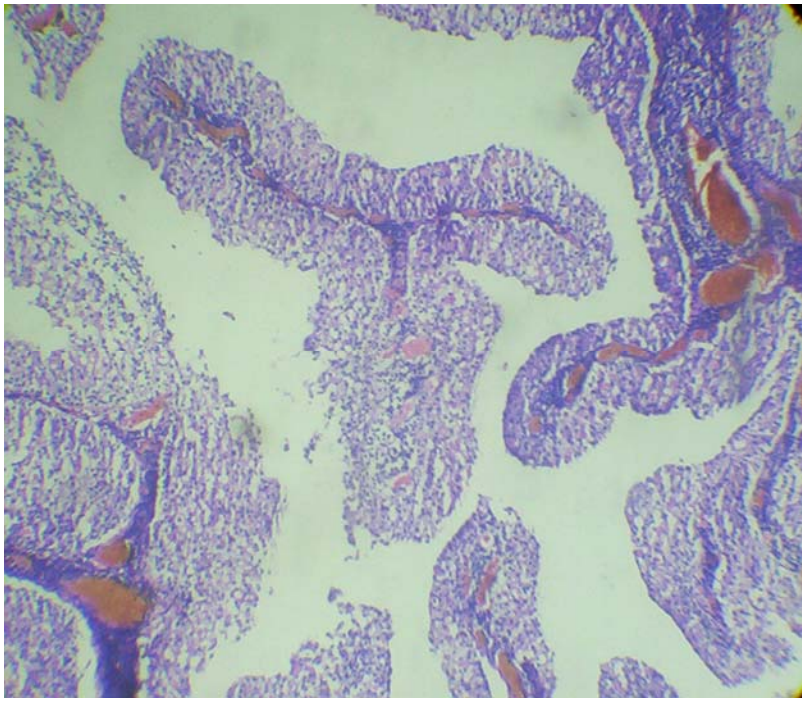
Urine sediment smear of low grade TCC. Pap stain 400X.



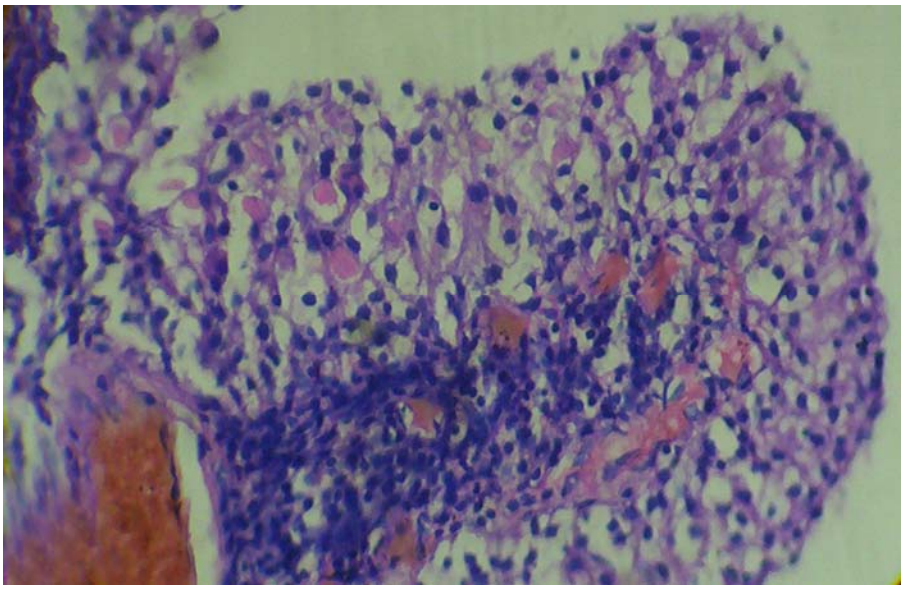
Urine sediment smear of low grade TCC H&E 400X



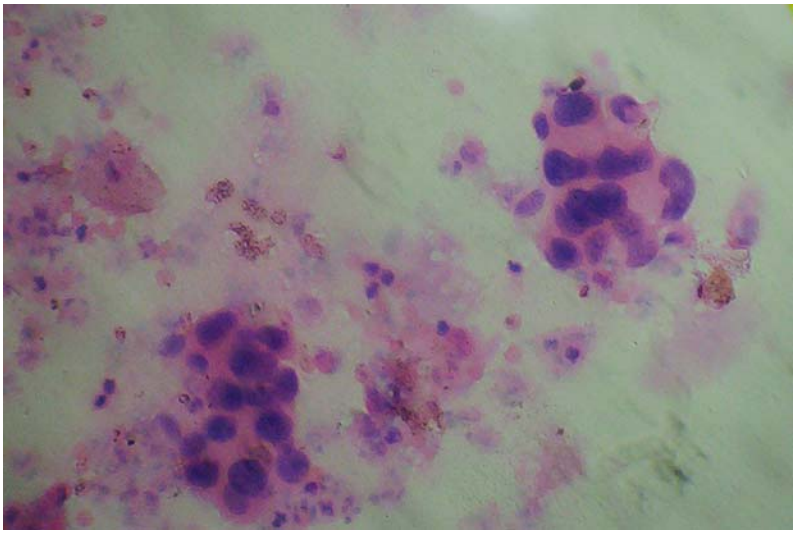
Histology of low grade papillary TCC showing branching, delicate papillae. Scanner view



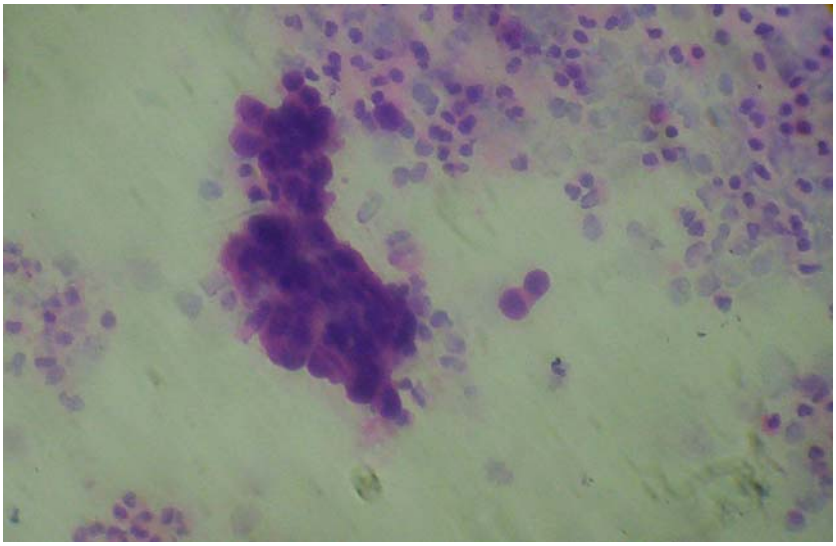
Histology of low grade papillary TCC showing branching, delicate papillae with orderly arrangement of cells H & E 100X.



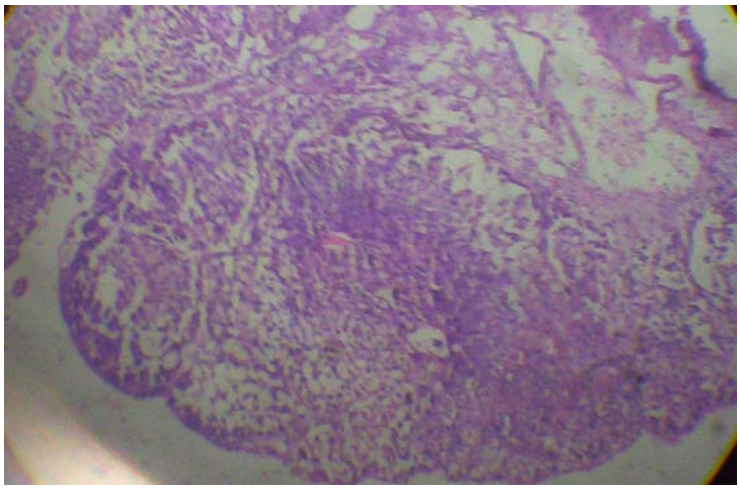
Histology of low grade papillary TCC showing papillary structure with orderly arranged cells. The cells show mild nuclear size variation H & E 400X.



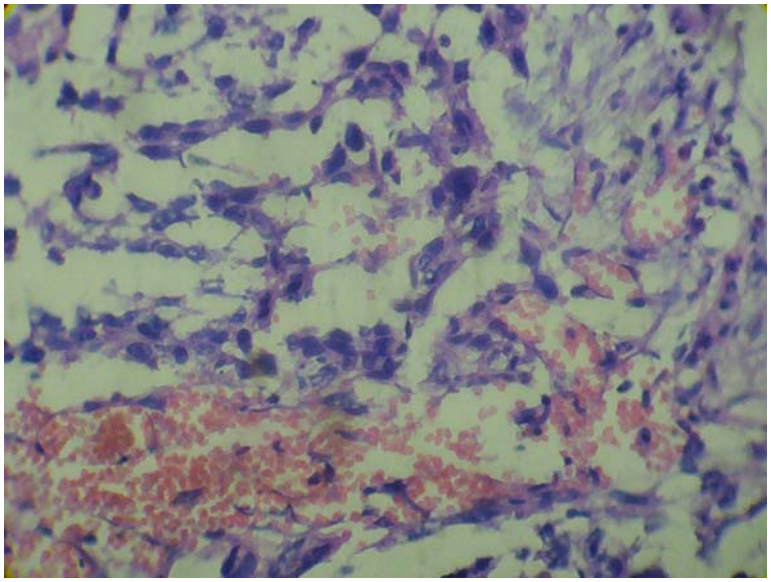
Urine sediment smear of high grade TCC showing large cells with pleomorphic hyperchromatic nuclei. H&E 400X.



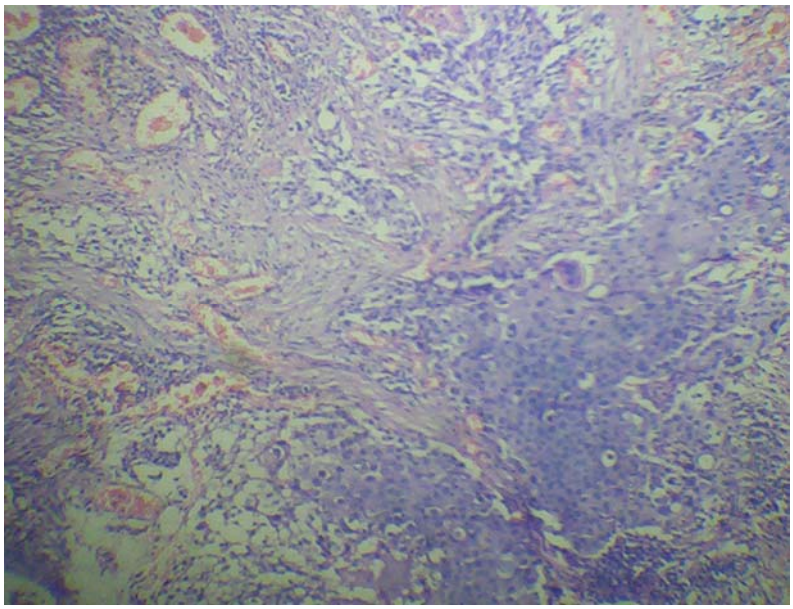
Urine sediment smear of high grade TCC showing large cells with pleomorphic hyperchromatic nuclei. H&E 400X.



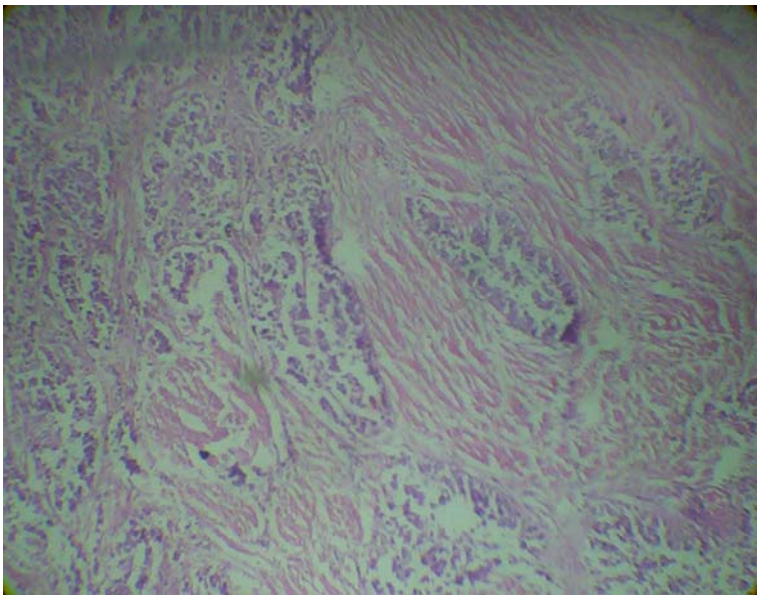
Histology of high grade papillary TCC H &E 100X.



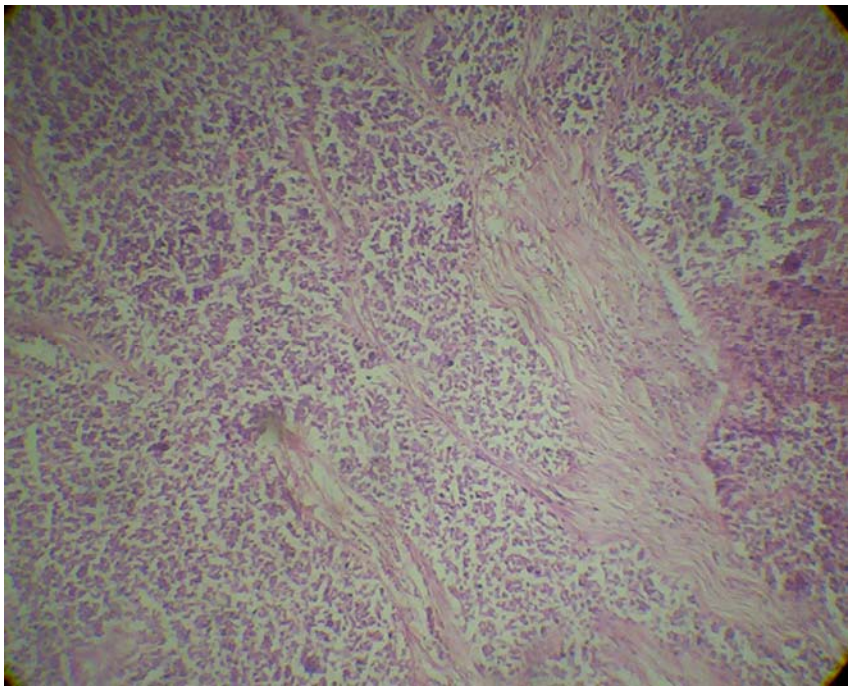
Histology of high grade papillary TCC showing cells with pleomorphic hyperchromatic nuclei H & E 400X



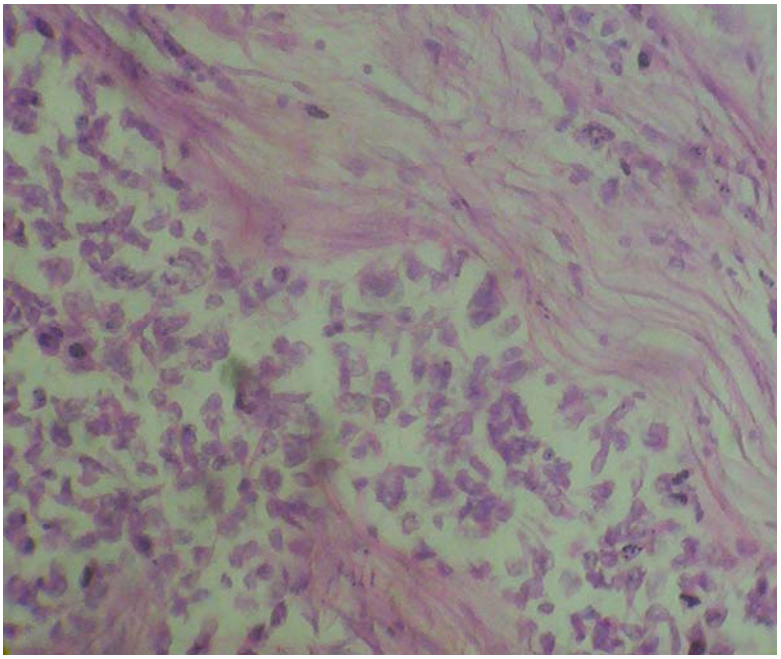
Histology of high grade papillary TCC with squamous metaplasia H & E 100X.



Histology of TCC with muscle invasion H & E 100X



Histology of TCC with muscle invasion H & E 100X



Histology of TCC with muscle invasion H& E 400X

High-grade transitional cell carcinoma

Loose clusters or isolated large cells with vacuolated cytoplasm, eccentricity placed pleomeorphic nuclei with increased N/C ratio [Nuclear Cytoplasmic ratio], irregular nuclear borders, coarse uneven chromatin and multiple nucleoli were diagnosed as high grade transitional cell carcinoma[50,51].

Cyto-hystological correlation was done patient based rather than specimen based. Such that one patient with multiple specimens, correlation was tabulated only one time.

A positive correlation satisfied one of the two conditions.

1. A patient with at least one positive urinary cytology concurrent with positive histological specimen or
2. A patient with consistently negative urinary cytology and histology specimen.

A discordant correlation was taken when at least one positive diagnosis by either cytology or histology and negative findings by the other modality.

An explanation of discordant results was determined.

RESULTS AND OBSERVATION

A total of 54 cases were studied for cyto-histological correlation of bladder cancer.

Table 1 shows age/sex wise distribution of 54 cases

TABLE 1:

AGE[Years]	MALE[Number]	FEMALE[Number]
20-29	2	
30-39	1	
40-49	7	1
50-59	19	1
60-69	18	
70-79	4	
80-89	1	
TOTAL	52	2

Among 54 cases, histologically proven bladder tumor cases were 45. Table 2 shows age/sex wise distribution of histologically proven cases.

TABLE 2

AGE[Years]	MALE[Number]	FEMALE[Number]
20-29	2	
30-39	1	
40-49	7	1
50-59	15	
60-69	14	
70-79	4	
80-89	1	
TOTAL	44	1

Among 54 cases, nine cases showed histologically negative results.

Table 3 shows cytological and cystoscopic findings in histologically negative cases.

TABLE 3

CASE NUMBER	HISTOLOGY	CYTOLOGY	CYSTOSCOPY
3	Negative	Negative	Thickening of bladder wall seen
21	Negative	Negative	? Growth right lateral wall of bladder.
22	Negative.	Negative	Growth right lateral wall
24	Negative	Positive	No growth
28	Negative	Negative	Bulging seen in the right lateral wall.
36	Negative	Positive	No growth
38	Negative	Positive	No growth
40	Negative	Positive	No growth
50	Negative	Negative	Thickened bladder wall seen

Histologic diagnosis and cytological findings of 54 cases was shown in table 4.

TABLE 4:

HISTOLOGY	CYTOLOGY		TOTAL
	POSITIVE	NEGATIVE	
LOW GRADE	11	10	21
HIGH GRADE	19	5	24
NEGATIVE	4	5	9
TOTAL	34	20	54

Cyto-Histological grading of urothelial neoplasm was shown in TABLE 5

TABLE 5:

HISTOLOGY	NUMBER OF CASES	CYTOLOGY	
		LOW-GRADE	HIGH-GRADE
LOW-GRADE	11	10	1
HIGH-GRADE	19	3	16

Cyto-Histological correlation for patients with bladder cancer shown in

TABLE 6.

TABLE 6 :

HISTOLOGICAL GRADE	TOTAL NUMBER OF CASES	CYTOLOGY		% OF POSITIVE
		POSITIVE	NEGATIVE	
LOW GRADE	21	11	10	52.38
HIGH GRADE	24	19	5	79.16

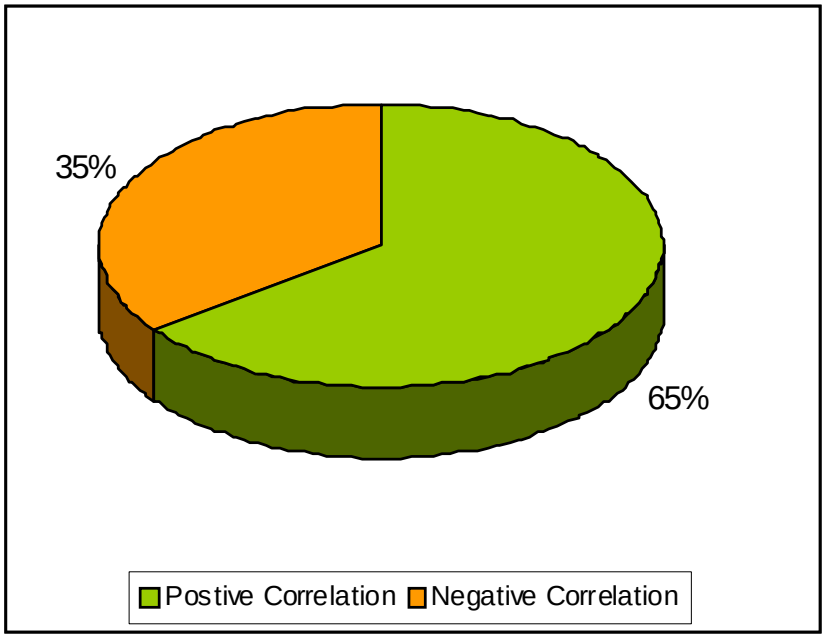
False positive findings on cytologic examination with clinical findings shown in

TABLE 7.

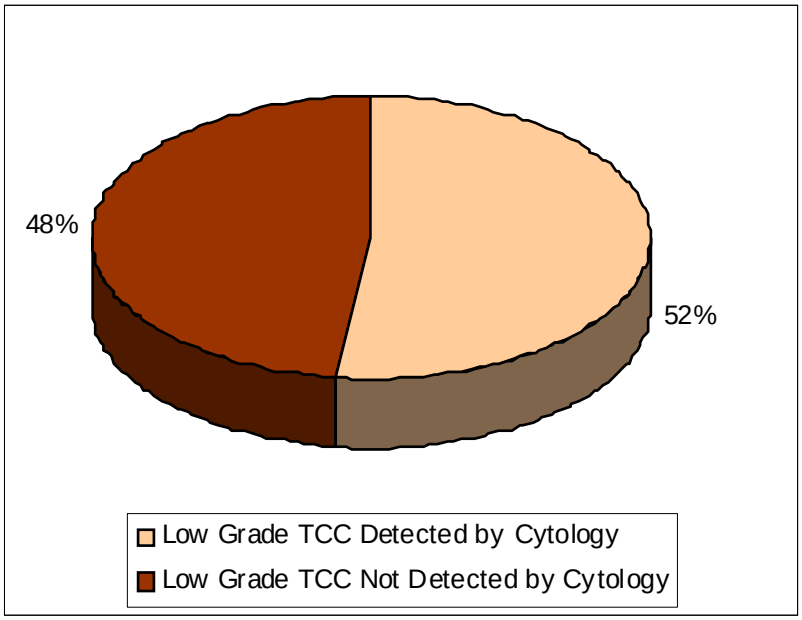
TABLE 7:

CASE NUMBER	CYTOLOGY	CLINICAL FINDINGS
24	Positive-High grade	Cystoscopy negative. Biopsy negative.
36	Positive -High grade	Cystoscopy negative. Biopsy negative. History of radiation present.
38	Positive-High grade	Cystoscopy negative. Biopsy negative
40	Positive-High grade	Cystoscopy negative.Biopy negative.History of radiation present.

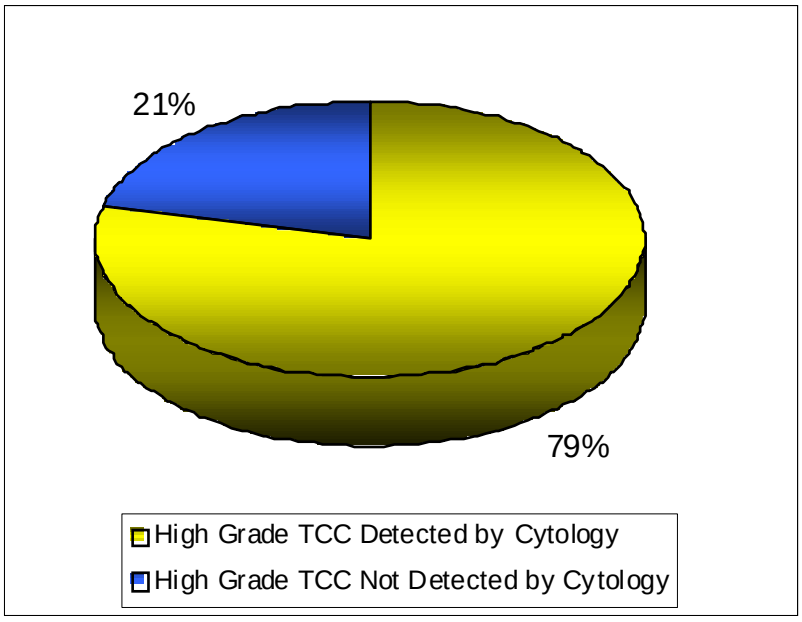
Overall, Cyto-Histological correlation was 65%



52% of the low-grade tumors were detected by cytology.



79% of the high-grade tumors were detected by cytology



Among the 54 cases

True positives [TP] were 30

False positives [FP] were 4

False negatives [FN] were 15

True negatives[TN] were 5

TABLE 8 shows these details.

TABLE 8

	BIOPSY		**TOTAL**
CYTOLOGY	POSITIVE	NEGATIVE	
POSITIVE	30[TP]	4[FP]	34
NEGATIVE	15[FN]	5[TN]	20
TOTAL	45	9	54

Specificity of urine cytology= TN/TN+FP=56%

Sensitivity of urine cytology=TP/TP+FN=67%

The results showed more number of bladder cancer cases in the age group of 50 - 69 years with male preponderance.

Among the 54 cases 45 were confirmed histologically, which includes 21 cases of low grade transitional cell carcinoma and 24 cases of high grade transitional cell carcinoma.

Among the 54 cases 9 were histologically negative.

28 out of 45 cases of transitional cell carcinoma were correctly identified by cytological examination.

2 cases showed suspicious cytology.

15 cases produced negative cytological findings.

Among the cytologically negative 15 cases, 10 were diagnosed as low grade tumors in histology and 5 were diagnosed as high grade TCC [Transitional cell carcinoma] in histology.

There were 4 false positives. **(Table 7)**

A summary of the cyto-histological grading results are shown in table 5. High grade TCC had high correlation whereas low grade TCC had a poor correlation.

Histologically confirmed high grade TCC occurred in 24 patients of whom 19 were detected cytologically i.e. 79% were detected by cytology.

Histologically confirmed low grade TCC occurred in 21 patients of whom 11 were detected cytologically i.e. 52% were detected by cytology.

Histologically confirmed TCC occurred in 45 patients of whom 30 patients were diagnosed cytologically i.e. 67% were detected by cytology.

Assessment of false positive cases:

Table 7 shows false positive cases i.e. positive cytology and negative histopathology diagnosis.

4 cases showed false positive cytological findings.

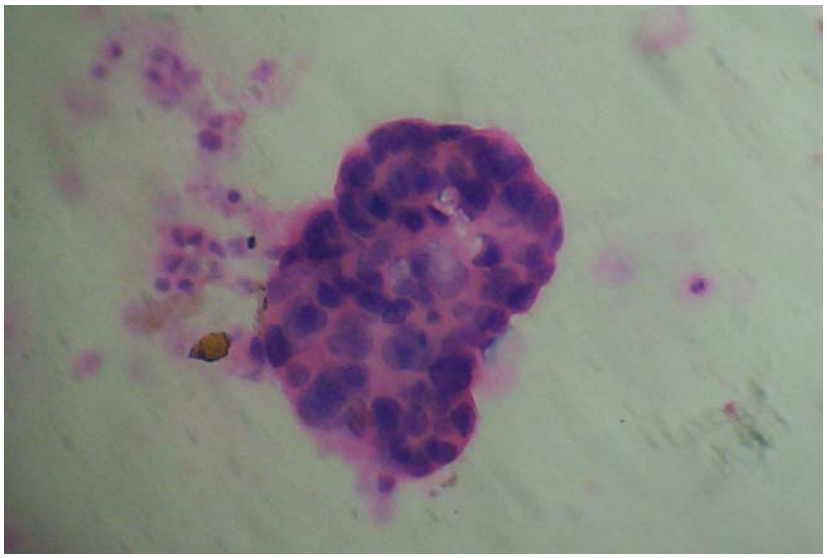
2 out of 4 cases received radiotherapy in the past and the radiation induced changes were falsely diagnosed as high grade TCC. In 2 cases, the cytology was positive and cystoscopy showed no growth. Random biopsy was negative for transitional cell carcinoma. Thereafter these two patients could not be followed up.

Assessment of false negative cases:

15 cases showed false negative cytology i.e. cytology negative and histology positive.

Among the 15 cases 10 cases were diagnosed as low grade tumor by histology and 5 were diagnosed as high grade tumor by histology.

Among the 10 low grade tumors, which were cytologically negative, 4 showed atypical and degenerative changes and 6 showed only normal urothelial cells.



Urine sediment smear; catheter artifact- showing papillary clusters of cells with smooth outline H&E stain 400X.

Among the 5 high grade tumors, which were cytologically negative, 3 showed atypical changes. Among these 3 which showed atypical changes, 2 showed abundant background acute inflammatory cell collection.

During our study we came across one urinary specimen smear which showed papillary clusters with smooth borders; it was due to previous catheterization.

DISCUSSION

A cytological examination of voided urine is the only non invasive and totally harmless method of detection, diagnosis and follow up of tumors of the lower urinary tract. Bladder washing or barbotage is an invasive procedure. In our study voided urine samples from the patients were used for cytological examination.

The value of repeated cytological examination of urine for 3 consecutive days was reported by Leopold G. Koss et al 1985[24]. In the present study also urine was examined on 3 consecutive days.

The average patient is 65 to 70 years old and most patient are older than 50 years In our study maximum number of cases (29) occurred in the age group of 50 - 69 years and total number of patients over 50 years of age was 34.

Consistent with previously published data[13,14,24,44] in the literature, this study also showed higher diagnostic accuracy with high grade tumors and low with low grade tumors. In our study 79% high grade tumors were detected by cytology but only 52% of the low grade tumors were detected by cytology.

Cigarette smoking is a risk factor for bladder cancer. In our study, of the 45 biopsy proven bladder cancer cases, 36 cases occurred in smokers.

Eighty percent of the patients with bladder carcinoma present with gross or microscopic hematuria[21]. In our study hematuria is a presenting symptom in 38 out of 45 biopsy proven bladder cancer patients.

Cystoscopy and cytology are complementary studies. The low grade papillary

lesions that may be difficult to diagnose cytologically are usually easy to diagnose

cystoscopically.In our study out of 10 cases which were falsely diagnosed as negative in cytological examination 8 showed cystoscopically diagnosable growth.

The sensitivity and specificity of the voided urine cytology in the studies conducted by various authors.

S.NO	Author	Sensitivity	Specificity
1.	M.N.EL-Bolkainy[30] 1980	44.7 to 97.3% with mean of 73.8%	88% to 99.5% with mean of 97.1%
2.	Uma A. Shenoy et al[44] 1985	85%	99%
3.	Asitava Mondal et al[4] 1992	92%	88%
4.	Misra V et al[28] 2000	47.37%	41.18%
5.	V. Poulakis et al[46] 2001	62%	96%
6.	A. Saad et al[3] 2002	48%	87%
7.	Planz B et al[35] 2005	38%	98.3%

Our study showed a sensitivity of 67% and specificity of 56% for urine cytology.

The study conducted by William M. Murphy et al[50] 1984-showed overall cyto-histological correlation of 92%

The study conducted by Uma A. Shenoy et al[44] 1985-showed overall cyto-histological correlation of 74%

In the study conducted by DiBonito L et al[7] 1992 cyto-histological correlation varied from 20% to 92.8%.

In our study the overall cyto-histological correlation was 65%.

False positive cases:

Radiation can cause cellular changes like multinucleation, nuclear enlargement, cytoplasmic vacuolation, Karyorrhexis.

The study conducted by P. N. Cowen[36] showed that it is impossible to state with any degree of certainty whether any atypical or bizarre cells seen in urine indicate a neoplastic origin or whether they are due to the irradiation of non-neoplastic epithelial cells.

Our study also showed similar findings. In two cases, who previously received radiation, the urine cytology showed bizarre cells which were diagnosed falsely as high grade TCC [Transitional cell carcinoma].

So it is essential that the cytologists be informed whether (and when) previous radiotherapy has been given to the patient as insisted by P. N.Cowen[36].

In 2 cases, the cytology was positive and cystoscopy showed no growth. Random biopsy was negative for transitional cell carcinoma. Thereafter these 2 Patients could not be followed up. In-adequate follow-up may be the cause for false-positivity.

According to David M. Schwalb et al[6], inadequate follow up may be the cause of false-positivity, he also said that a positive and even suspicious result in the absence of visible disease in the previously untreated patient predicts the eventual development of clinically recognizable transitional cell carcinoma, mainly in the bladder.

False positive rates reported by various authors:

The study conducted by M.N.EL-Bolkainy[30] showed false positive rate of 1.5%. The study conducted by Uma A. Shenoy et al[44] showed false positive rate of 11%. According to Edward M. Messing[8] it was 1-12%.

In our study it is 12%, it also correlates well with other authors' study.

False positive cytological findings may develop a tumor in subsequent years and therefore needs repeated examination of urine for malignant cells[4,31,44].

False negative cases:

Among the 45 biopsy proven bladder neoplastic cases 15 were falsely diagnosed as negative cytologically. Among the15 cases 10 were low grade tumor and 5 were high grade tumors.

Among the 10 low grade tumors, which were cytologically negative 6 showed only normal urothelial cells and 4 showed atypical and degenerative changes. This may be due to exfoliation of non-diagnostic cells from low grade tumors.

Among the 5 high grade tumors which were cytologically negative, 3 showed atypical cellular changes. Among the 3, two showed heavy background inflammatory cell collection. 2 out of 5 high grade tumors didn't show any malignant cells in cytology. This may be due to masking of the tumor cells by associated inflammation or hemorrhage, and scanty exfoliation of necrotic tumors as stated by M.N.EL-Bolkainy[30].

Three case of histologically proven high grade tumors were detected as low

grade tumors by cytology and one case of histological low grade tumor was cytologically diagnosed as high grade tumor, a more likely explanation is that the bladder cancers are rarely composed of a pure population of cells, all of which are in the same stage of differentiation at any one point in time. High grade TCC may exhibit surface maturation, and tumor cells exfoliated from these areas could have a cytological features of a low-grade neoplasm, less commonly small areas of anaplasia may exist within predominantly low grade tumors.

LIMITATIONS

- As the sample population is small the sensitivity and specificity of this study may not be so accurate.

- Even though urinary telomerase activity is the most sensitive and specific test to detect bladder tumors we have not done it because of its cost.

- As the study is time bound we are not able to detect the time interval between primary diagnosis and recurrence.

SUMMARY

- More than 50% (64.44 %) of cases occurred in the age group of 50 - 69 years with male preponderance
- More than 50% (67%) of the bladder cancers can be detected by simple, non-invasive, voided urine cytology.
- Nearly 80% (79%) of the high grade bladder cancers can be detected by voided urine cytology.
- But only nearly 50% (52%) of the low grade bladder cancers can be detected by voided urine cytology.
- More than 50% (65%) of the cytological diagnosis were correlated well with histopathological diagnosis.
- Inadequate follow up and previous therapy led to false positive diagnosis.
- Cystoscopy is more useful in low grade tumors which were diagnosed falsely as negative by cytology.

CONCLUSION

- Cytological examination of urine specimen is valuable as an aid in the diagnosis and follow up study of bladder tumors.
- The voided urine cytology is not only of diagnostic but also of prognostic value; positive cytology presumably identifies patients at high risk.
- The accuracy is more with high grade tumors.
- Voided urine cytology correlates well with histological diagnosis in more than 50% cases
- Previous therapy reduces the diagnostic accuracy of voided urine cytology.
- Cystoscopy may be of more useful in diagnosing low grade tumors which were missed by voided urine cytology.
- Voided urine cytological study can be a most valuable adjunct to the clinician in the evaluation of urologic patient as it is simple, non-invasive with good accuracy in the diagnosis.

Master Chart

Case No	Name	Age/Sex	IP.No	Cytological Diagnosis					Histopathological Diagnosis			
				C.No	Positive Grade		Negative	Suspicious	B.No	Positive Grade		Negative
					Low	High				Low	High	
1	Mr. Mohan	26/M	684210/04	5109/04			*		5761/04	*		
2	Mr. Kamaraj	46/M	685552/04	5341/04		*			6424/04		*	
3.	Mr. Murugan	55/M	684773/04	5407/04			*		6152/04			*
4.	Mr.Sambath	45/M	684745/04	5408/04				*	6060/04	*		
5.	Mr.Murugayan	57/M	685395/04	5492/04		*			6151/04		*	
6.	Mr Duraikannan	65/M	689193/04	6042/04			*		6693/04	*		
7.	Mr.Durai.	50/M	696753/04	6571/04		*			7282/04		*	
8.	Mr. Sadagopan	69/M	700618/05	1/05			*		186/05		*	
9	Mr. Varadhan	72/M	704264/05	471/05	*				482/05	*		
10.	Mr.Thanikachalam	50/M	707349/05	834/05		*			955/05		*	
11.	Mr.AdiNarayanan	46/M	708362/05	929/05		*			934/05		*	
12.	Mr.Sethan	61/M	708453/05	946/05	*				1014/05	*		
13.	Mr. Lingam	55/M	709585/05	1024/05		*			961/05		*	
14.	Mr.Duraiswamy	44/M	710411/05	1142/05				*	1128/05	*		
15	Mr.SathyaNarayanan.	65/M	704270/05	1226/05			*		1035/05	*		
16	Mr.Krishnan	63/M	710102/05	1376/05			*		933/05		*	
17	Mr.Kasi	60/M	712379/05	1411/05	*				2080/05		*	
18	Mr.Kalyani	50/M	712348/05	1412/05	*				1671/05		*	
19	Mr. Madhar	45/M	713183/05	1421/05			*		1558/05		*	
20	Mr Arumugam	47/M	710084/05	1438/05		*			1327/05	*		
21	Mrs. Saroja	50/F	714274/05	1506/05			*		1223/05			*
22	Mr.Bhatacharji	68/M	714423/05	1629/05			*		1490/05			*
23	Mr.Mani	70/M	716892/05	1883/05		*			1602/05		*	
24	Mr. Kaveri	68/M	716878/05	1895/05		*			1815/05			*
25	Mr. Kannan	60/M	717720/05	2038/05		*			1763/05		*	
26	Mr.Sengai.	59/M	715917/05	2976/05			*		1628/05	*		
27	Mr.Thangavel	65/M	718966/05	2165/05			*		1963/05		*	
28	Mr. NavajNivas	59/M	720086/05	2166/05			*		1967/05			*

29	Mr. Kadupadi	52/M	720549/05	2406/05		*			2101/05		*	
30	Mr. Santhanam	64/M	724401/05	3044/05		*			2723/05		*	

Case No	Name	Age/Sex	IP.No	Cytological Diagnosis					Histopathological Diagnosis			
				C.No	Positive Grade		Negative	Suspicious	B.No	Positive Grade		Negative
					Low	High				Low	High	
31	Mr. Venkatamuni	65/M	726712/05	3099/05		*			2719/05		*	
32	Mr. Chinnakannu	55/M	724603/05	3128/05		*			2741/05		*	
33	Mr. Gandhimathinathan	49/M	639747/05	3944/05	*				2989/05	*		
34	Mr. Kaliappan	65/M	732583/05	4061/05	*				4129/05	*		
35	Mr.Balakrishnan	54/M	734048/05	4064/05		*			3619/05		*	
36	Mr. Elumalai.	51/M	750184/05	4075/05		*			5041/05			*
37	Mr. Sundaram.	65/M	753638/05	4083/05			*		5140/05	*		
38	Mr. Devarajan	55/M	733837/05	4085/05		*			5970/05			*
39	Mr. Venugopal	85/M	734863/05	4195/05		*			3620/05		*	
40	Mr. Ramachandran	60/M	736076/05	4199/05		*			3914/05			*
41	Mr. Kuppan.	65/M	737702/05	4440/05			*		4206/05	*		
42	Mr. Subramani	28/M	743201/05	4717/05			*		3802/05	*		
43	Mr. Periyasamy	50/M	743538/05	4985/05	*				4727/05	*		
44	Mr. Velu	60/M	745407/05	5185/05	*				4542/05	*		
45	Mr. Veeraswamy	58/M	745207/05	5251/05	*				4436/05	*		
46	Mrs.sarojini	40/F	752349/05	5973/05		*			5068/05		*	
47	Mr. Durairajan	51/M	753042/05	6212/05	*				5454/05	*		
48	Mr.Muthu	70/M	754780/05	6331/05			*		5479/05	*		
49	Mr. Kadharbasha	52/M	755680/05	6469/05			*		5481/05		*	
50	Mr. Govindaswamy	52/M	765278/05	6853/05			*		6088/05			*
51	Mr. Natarajan	69/M	764855/05	7397/05		*			6254/05		*	
52	Mr. Babu	50/M	770398/05	8000/05			*		6748/05	*		
53	Mr.Muthunadar	70/M	773171/05	8336/05			*		290/06		*	
54	Mr. Ganesan	36/M	782218/06	738/06			*		777/06	*		

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