Original Article

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Pathology Section

Role of Wet Mount and Cytospin Smears in Diagnosing Urothelial Carcinoma: A Descriptive Observational Study

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ABSTRACT

Introduction: Most common carcinomas of lower urinary tract are of urothelial type in which, majority of them occur in the bladder. The demonstration of exfoliated neoplastic cells in urine sediment cytology is one of the choices of investigation.

Aim: To study the morphology of atypical cells of urine sediment in unstained wet mount preparation and urine sediment obtained by cytospin smear stained with Papanicolaou stain.

Materials and Methods: It was a descriptive observational study done over a period of 29 months i.e., from July 2016 to December 2018 at SS Institute of Medical Sciences and Research Centre, Davangere, Karnataka, India. Using non probability sampling method, 50 urine samples were analysed by DIRUI-H 500 a semi automatic urine analyser, which were further examined by wet mount preparation and cytospin smear stained with papanicolaou stain. Results were analysed by whether the smears were positive or negative for atypical cells based on the cytological and nuclear details.

Results: The yield of Red Blood Corpuscles (RBCs) was significantly increased in cytospin preparation with morphology of RBCs was better appreciated in wet mount compared to cytospin preparation. The yield of atypical cells was significantly increased in cytospin preparation with the morphology of atypical cells were better appreciated in pap stained cytospin preparation compared to wet mount urine examination.

Conclusion: Overall, cell yield and preservation of White Blood Corpuscles (WBCs), epithelial cell and atypical cells morphology was better in cytospin preparation, while RBCs were better appreciated in wet mount preparation. Cytospin technology is a quick, efficient and cost effective method for increasing cell yield in less cellular samples and also helps in providing better cellular morphological details.

Keywords: Papanicolaou stain, Urinary tract, Urine analysis, Urine sediment cytology

INTRODUCTION

Routine urine analysis is composed of two parts; the dipstick to measure several analytes and microscopic examination of the urine sediment. Dipstick results determine the need for urine sediment cytology [1]. An accurate and careful examination of urine sediment is a best indicator of status of the renal and genitourinary system [2]. As it allows detection of diverse elements viz., cells with nuclear atypia, casts and crystals associated with varied pathologies [2-4]. Urine cytology is a primary screening and surveillance modality for the detection of high grade urothelial neoplasia with a specificity of 90% [5-7]. Cytospin preparations are relatively superior in preserving cytomorphologic details, architectural patterns and provide better cell yield thereby, contributing to improve the diagnostic accuracy in urine cytopathology [8-10]. The founders of Ayurveda, Charaka and Sushruta during the sixth century had noticed that large black ants were attracted to diabetic urine [4,5]. Hippocrates, (460-377 BC) had used urine as a means of diagnosis and examination of human body functions [6,7].

Serum and biochemical parameters do not show any abnormalities until late stage of renal disease. The urine sediment examination has immense importance in early detection of renal disease as it is simple and cost effective. Hence, it is known as "liquid renal biopsy" [11,12]. Analysis of urine, is one of the most common investigation not only for diagnosing renal and urinary tract disease but also for metabolic or systemic diseases [13,14]. The basic components of urine analysis consists of four parts: specimen evaluation, physical examination, chemical examination and microscopic examination of urine sediment [2,14]. Urine should be collected in a clean container, stored in cool place and tested as soon as possible [2,15].

The present study was undertaken to observe and study the morphology of atypical cells of urine sediment in unstained wet mount preparation and urine sediment obtained by cytospin smear stained with papanicolaou stain.

MATERIALS AND METHODS

A descriptive observational study was conducted in SS Hospital and Research Centre, Davangere, Karnataka, India, over a period of 29 months from July 2016 to December 2018 after taking permission from the Institutional Ethical Committee (IERB ref no 702016/38-2016).

Inclusion criteria: Urine sediment obtained from urine samples of patients who were referred to the laboratory for urine analysis, presented as bladder mass on Ultrasonography (USG)- reported by the radiologists were included.

Exclusion criteria: The samples less than 10 mL, collection bag or from catheter, paediatric cases, bacteria, yeast and non cellular elements on microscopy were excluded from the study.

Sample size calculation: By using non probability sampling method 50 urine samples of both sexes, obtained in the laboratory for urine analysis within the study period were included as sample size.

Study Procedure

The patient's freshly voided 20-25 mL of urine was collected. Samples were centrifuged at 1000 rpm for three minutes then a drop of the sediment was pipetted on a microscope slide, cover slipped and observed under bright field microscope. A 450 microlitres of this sample was taken for making cytospin smears using cellspin 1 (Thermac) which were fixed in 95% ethanol and stained with papanicolaou stain. Atypical cells were classified as negative or positive and structural details were noted.

Microscopic Examination of Urine Sediment

All the 50 urine samples included for the study were examined microscopically both on wet mount preparation and pap stained cytospin smears.

The microscopic sediment was observed for cellular and non cellular constituents. Cellular elements were classified as RBCs, WBCs, epithelial cells and atypical cells. Non cellular constituents included casts and crystals. Initial microscopic study was done on wet mount unstained preparation of urine sample. The observations were recorded. This was followed by microscopic examination of pap stained cytospin smear. These microscopic findings were also recorded separately.

STATISTICAL ANALYSIS

Data were recorded in excel sheet and analysed using descriptive statistics. EPIINFO version 6 software was used for calculation. Microscopic findings by both methods were correlated by Cohen's kappa coefficient, describing about the presence or absence of atypical cells.

RESULTS

In the present study, age of the patients ranged from 60-80 years, with mean age of 67.21 years. A 35 (70%) urine samples were from males (M) and 15 (30%) were from females (F), with M:F ratio of 2.2:1 [Table/Fig-1].

Age in years	Male (n)	Female (n)
60-70	12	06
71-80	23	09
Total	35	15

[Table/Fig-1]: Age and sex distribution of the present study.

Volume: The volume of the urine samples ranged from 20-25 mL.

Colour: In the present study 39 urine samples were red (haematuria), six were yellow and, five sample was turbid in colour.

pH: pH of the urine sample varied. In 34 samples, pH was between 5.5-6, and 14 samples had pH of <5.5. One sample each had a pH of >6.5 (0.5%) and >7.5 (0.5%).

Specific gravity: Specific gravity ranged from 1.010-1.025 in 36 urine samples. Specific gravity was >1.025 in 13 (46.5%) samples. Specific gravity was <1.010 (0.5%) in one sample.

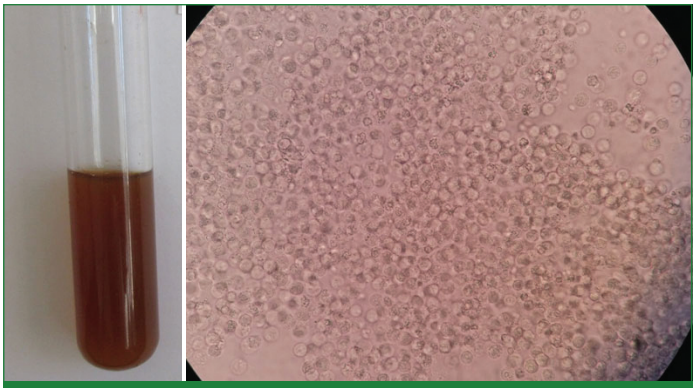
Number of urine samples with abnormal chemical constituents was discussed in [Table/Fig-2].

Abnormal chemical constituents	Number of urine samples	Percentage (%)
Protein	22	44
Glucose	15	30
Ketone	10	20
Bilirubin	10	20
Urobillinogen	7	14
RBCs	48	96
Nitrite	35	70
Pus cells	40	80

[Table/Fig-2]: Number of urine samples with abnormal chemical constituent-automated analyser.

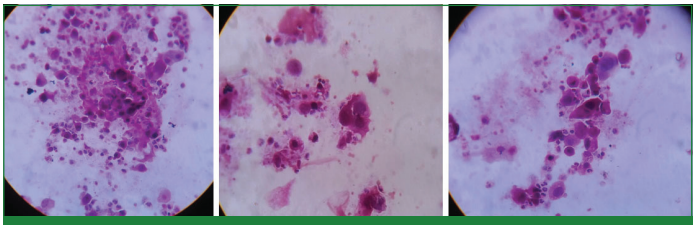
Atypical cells in urine sediment: Atypical cells in the urine sediment were recorded as present or absent after microscopic examination. Urine sediment from 48 samples showed atypical cells, 2 samples were negative for atypical cells. 39 samples showed gross haematuria [Table/Fig-3].

Wet mount preparation: In the present study, 50 samples showed few epithelial cells of slightly varying size in a background of dense population of RBCs. Cellular details were not made out [Table/Fig-4].



[Table/Fig-3]: Gross haematuria.
[Table/Fig-4]: Wet mount smears- plenty of RBCs (40x). (Images from left to right)

Cytospin preparation: Pap stained smears from the 48 samples showed clusters as well as single cells with anisonucleosis and pleomorphism. The cells were predominantly round to oval with dense eosinophilic cytoplasm and hyperchromatic nuclei. The remaining two samples showed plenty of pus cells [Table/Fig-5].



[Table/Fig-5]: Cytospin smear- clusters of atypical cells (Pap, 40x).

As mentioned in the [Table/Fig-6], 48 urine samples showed atypical cells on pap stained cytospin smears with nuclear and cytoplasmic details where on wet mount authors were able to see only pus cells and RBCs. The Cohen's kappa coefficient was 0.078 which was calculated for correlating cellular morphology of atypical cells in wet mount and cytospin smears. This value (0.078) indicates wet mount smears have poor value in predicting atypical cells, whereas cytospin smears were good predictors for atypical cells.

Cytomorphological details	Number of samples with cytoplasmic margin	Cytoplasmic details and nuclear details	Differential staining of cytoplasm
Wet mount	Nil	Nil	Nil
Pap stained cytospin smear	48 (100%)	48 (100%)	48 (100%)

[Table/Fig-6]: Cytomorphological details of atypical cells in wet mount and pap stained cytospin smear (n=48).

DISCUSSION

Urine sediment examination is of high diagnostic value in patients with high grade carcinoma of the urinary bladder [16]. Morphology of atypical cells was better appreciated in pap stained cytospin preparation compared to wet mount urine examination. Nuclear characteristics viz., nuclear margin, hyperchromatism, coarse chromatin, nucleoli were very clearly observed in pap stained smear of cytospin preparation along with cytoplasmic characteristics, high nucleo-cytoplasmic ratio and bizarre nuclei. Definitive cytological diagnosis of malignancy was not possible on wet preparation these findings were correlated by the other studies. Of the 50 urine sediments studied, more samples were from males with M:F ratio of 2.2:1. This is insignificant since non probability sampling method was used. M:F ratio of patients included in other studies like Bhagyalakshmi A et al., Mambatta AK et al., also showed a slight male preponderance [Table/Fig-7] [11,17-20].

Mean age of the patients was 67.21 years with 32 (64%) more samples belonging to patients in 7th and 8th decade. Colour of the urine sample varied but most of them were red 39 (78%). The colour of urine has been considered to be one of the most vital tools

Various studies	M:F
Bhagyalakshmi A et al., (2014) [11]	1.4:1
Saimary AL et al., (2006) [18]	1.2:1
Wald R et al., (2009) [19]	1.5:1
Renshaw A (2000) [20]	1.7:1
Mambatta AK et al., (2015) [17]	1.3:1
Present study	2.2:1

[Table/Fig-7]: Sex distribution- comparative studies [11,17-20].

for assessment of hydration. Abnormal number of RBCs in urine is known as haematuria (>3 RBCs/hpf) [2]. The urine microscopy is most sensitive method for detecting haematuria [21]. Most common cause of haematuria is trauma to urinary tract. The other causes for haematuria include both neoplastic and non neoplastic lesions of kidneys or urinary tract, bleeding disorders and use of anticoagulants. Methods used to detect blood in urine are benzidine test, orthotoluidine test and haemastrip test [22].

The normal pH of urine ranges from 4.6-8. pH of more than half of samples included for the study was in the range of 5.5 to 6 (n=34, 68%). Normal kidneys have the ability to produce urine with specific gravity ranging from 1.003-1.035 [23]. Microscopy of urine sediment is a simple and inexpensive, but often overlooked procedure, it can contribute to swift, correct diagnosis of suspected kidney and urinary tract disease [19,24]. It is essential that microscopic examination should be done when the sample is fresh, particularly within 1-2 hours of collection. As cells and casts begins to lyse within two hours of collection. Take 15 mL of urine into test tube, centrifuge the tube at 2000 rpm for five minutes and discard the supernatant. A drop of sediment is placed on a slide. Cover it with a cover slip for microscopic examination of urine [25]. Renal epithelial cells are the single layer of cells lining the nephron these include cells lining the glomerulus, the proximal and distal convoluted tubules and the collecting ducts. Recognition of renal epithelial cells is difficult, especially in the wet urine sediment and morphologic characteristics vary depending on the place of origin within the nephron. They are especially difficult to distinguish from the small forms of transitional epithelial cells (urothelium) [26]. Morphology of epithelial cells was better appreciated in pap stain cytospin preparation compared to wet mount urine examination. On pap stained cytospin preparation both the nuclear and cytoplasmic details of epithelial cells were well appreciated. Pap stain gave better nuclear morphology and the differential cytoplasmic stain enabled in differentiating the epithelial cells and to categorise them.

The meticulous observation of fresh urinary sediments allows the identification of diverse cellular types associated with varied pathologies [13]. The primary objective of urinary cytopathology is to detect and diagnose high-grade urothelial carcinoma [14]. Urinary cytology is used to monitor patients with a history of urothelial neoplasms [15]. High-grade urothelial tumours on pap stained urine sediment cytology shows clusters of neoplastic cells with hyperchromatism, multinucleation, macronucleoli and signs of cannibalism [15]. Atypical cells identification was difficult on wet mount preparation. The yield of atypical cells was significantly increased in cytospin preparation as supported by statistical analysis with Cohen's Kappa coefficient was 0.078 which indicates poor correlation. Morphology of atypical cells was better appreciated in pap stained cytospin preparation compared to wet mount urine examination. Nuclear characteristics viz., nuclear margin, hyperchromatism, coarse chromatin, nucleoli were very clearly observed in pap stained smear of cytospin preparation along with cytoplasmic characteristics, high nucleo cytoplasmic ratio and bizarre nuclei. Definitive cytological diagnosis of malignancy was not possible on wet preparation.

Deshpande V and Mckee G conducted a retrospective study on atypical cells in urine sediment using thin prep technique and found that specificity of urine cytology for transitional cell carcinoma was >90% and inferred that atypical category in urinary cytology remains a wastebasket and includes both specimens that have a high like hood of a significant lesion and specimens without this possibility [6]. Straccia P et al., compared cytospin and liquid-based cytology in urine specimen and observed no significant difference in terms of sensitivity and specificity between these methods in cases with high-grade carcinoma on urine sediment cytological examination [27]. Cytospin preparations are relatively superior in preserving cytomorphologic details, architectural patterns and provide better cell yield thereby, contributing to improve the diagnostic accuracy in urine cytopathology [28]. In an economical and technical point of view, cytocentrifugation with disposable chambers remains the standard meticulous technique of urine sample analysis for molecular studies and follow-up of patients with renal graft rejection [29].

Limitation(s)

Limitations of the present study were on cytospin smear, authors were unable to grade the urothelial carcinoma and in excessive haemorrhagic samples it was difficult to assess the nuclear details.

CONCLUSION(S)

Cytospin technology is a quick, efficient and cost effective method for increasing cell yield in less cellular samples and also helps in providing better cellular morphological details. Cytospin preparation helped in appreciating details of malignant cells compare to wet mount preparation of urine. Hence, cytospin smear examination is advised for routine cytological examination of urine.

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