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Pathology

WET MOUNT URINE MICROSCOPIC EXAMINATION VERSUS CYTOSPIN SMEARS STAINED WITH PAPANICOLAOU STAIN- A DESCRIPTIVE OBSERVATIONAL STUDY.

Dr Asha Patil	Assistant Professor, Department of Pathology, M. R. Medical College Kalaburagi.
Dr Pratima P	Assistant Professor, Department of Pathology, S.N. Medical College Bagalkot.
Dr Rohini Mokhashi	Assistant Professor, Department of Pathology, M.R. Medical College Kalaburagi.

ABSTRACT **Background & Objectives:** Wet mount urine examination in a routine investigation done for diagnosing renal and urinary tract disease, metabolic and systemic disease. It is simple, and cost effective. This study is undertaken to observe and correlate urine sediment cytology by two different methods i.e. wet mount and Pap stained smears of cytocentrifuge preparation. **Methods:** It is a descriptive observational study conducted over a period of two years. Using non-probability sampling method, 200 urine samples were subjected for sediment cytology using wet mount preparation and Pap stained Cytospin preparation. **Results:** The yield of RBCs, neutrophils, epithelial cells and atypical cells was significantly increased in Cytospin preparation. Morphology of RBCs was better in wet mount where as morphological details of neutrophils, epithelial cells, and atypical cells were better appreciated in Pap stained Cytospin preparation. Wet mount was better for differentiating various casts.Wet mount was better method for observation for crystals. **Conclusion:** Both methods have their own advantages. Wet mount still appears to be the method of choice for casts and crystal in urine and RBC morphology. Pap stained Cytospin preparation not only yields more cells but also gives better morphological details of cells.

KEYWORDS : Urine sediment cytology, Cytospin smear, Papanicolaou stain.

INTRODUCTION

Urine sediment examination is time saving, cost effective and non-invasive method in diagnosing not only renal disease but also in case of systemic diseases like Diabetes mellitus, pre-eclampsia and inherited metabolic syndromes e.g.:- Phenyl ketonuria, Tyrosinemia, Alkaptonuria.[1]

An accurate and meticulous urine sediment analysis is a good indicator of status of the renal and genitourinary system, as it allows detection of diverse elements viz cells, casts and crystals associated with varied pathologies.[1]

Urinary sediment examination helps in detection of cellular and non cellular elements as observed in fresh or stained smears. Components of urine examination includes specimen evaluation, physical examination, chemical examination and microscopic examination of urine sediment.[2]

The microscopic examination of urine was established for the first time, at the beginning of sixth-seventh century by Zacharias Jansen and Nicolas Fabricus. They described urine crystals resembling "a heap of rhomboidal bricks" and referred to it as, uric acid crystals.[3,4,5] 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]

Urine cytology is a primary screening and surveillance modality for the detection of high grade urothelial neoplasia with a specificity of 90%.[6,7]

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]

This study is undertaken to observe and correlate, microscopic findings of unstained wet mount preparation of urine sediment and cytospin smears of urinary sediment obtained by cytocentrifugation and stained with Papanicolaou stain.

METHODOLOGY

The present research work on " **Wet mount Urine Microscopic examination versus Cytospin smears stained with Papanicolaou stain- A descriptive Observational study."** was undertaken in the department of pathology, SS Institute of Medical Science & Research Centre (SSIMS & RC), Davangere over a period of two years from July 2016 to June 2018.

Ethical permission for the study was obtained from Institutional Ethical Review Board (IERB) (*Annexure II*)

SOURCE OF DATA:

Urine sediment for the study was obtained from urine samples of patients attending either outpatient department or admitted under various specialties of S.S. Hospital and Research Centre, Davangere who were referred to the clinical pathology laboratory for urine analysis.

METHOD OF COLLECTION OF DATA:

10 – 30 ml of freshly voided random sample of urine from the patient was subjected for analysis.

Sample Size:

Using purposive sampling i.e. a non probability sampling method, 200 urine samples were included for the study which met the inclusion criteria.

- Pap stained smears were cover slipped and studied microscopically.
- Polarising microscopic examination was done wherever necessary.

Microscopic Examination Of Sediment: [1]

Both the wet mount preparation and Cytospin smear stained with Pap stain were observed microscopically.

RBCs, WBCs and epithelial cells were observed for better morphology.

Casts, crystals and atypical cells were classified as negative or positive and structural details were noted.

Exclusion Criteria:

Urine samples from the urine collection bag or indwelling catheter, stale sample, refrigerated sample, paediatric samples and inadequate samples (<10ml) were excluded from the study. Bacteria and yeast were excluded from the study.

Statistical Analysis:

Data were recorded in excel sheet and analysed using descriptive statistics.

EPIINFO VERSION 6 software was used for calculation. Cohen's Kappa coefficient was used to check agreement between wet mount urine sediment and Pap stained Cytospin smear.

RESULTS

Age And Sex Distribution

Age of these patients ranged from 20 years to 80 years, with mean age of 47.21 years. 113 (56.5%) urine samples were from males (M) and 87 (43.5%) were from females (F), with M: F ratio of 1.2:1.

Table 2: Age And Sex Distribution

Age Range (In Years)	Male		Female		Total	
	Number	%	Number	%	Number	%
20-29	20	(10%)	27	(13.5%)	47	(23.5%)
30-39	17	(8.5%)	14	(7%)	31	(15.5%)
40-49	16	(8%)	10	(5%)	36	(13%)
50-59	20	(10%)	12	(6%)	32	(16%)
60-69	22	(11%)	14	(7%)	36	(18%)
70-79	15	(7.5%)	10	(5%)	15	(12.5%)
>80	3	(1.5%)	0	(0%)	3	(1.5%)
Total (n=200)	113	(56.5%)	87	(43.5%)	200	100

Volume: The volume of these urine samples ranged from 15ml to 25ml.

Color

104 (52%) urine samples were pale yellow, 44 (22%) were red in color and 49 samples (24.5%) appeared yellow, one sample was turbid and 2 (1%) were orange in color.

pH

pH of the urine sample varied. In 110 (55%) samples pH was between 5.5 to 6, and 88 (44%) sample had pH of <5.5. One each sample had a pH of >6.5 (0.5%) and >7.5 (0.5%).

Specific Gravity

Specific gravity ranged from 1.010 to 1.025 in 106 (53%) urine samples. Specific gravity was >1.025 in 93 (46.5%) samples. Specific gravity was <1.010 (0.5%) in one sample.

Using purposive sampling method, 200 urine samples with presence of abnormal chemical constituents one or more than one detected on automated analyzer were included for the study.

Correlation Of Cellular Morphology In Wet Mount And Pap Stained Cytospin Preparation

Table 21: Cytomorphological Details Of Neutrophils In Wet Mount And Pap Stained Cytospin Preparation (n=120).

Cytomorphological details of neutrophils	Number of samples Cytoplasmic margin appreciated	Cytoplasmic details made out	Differential staining of cytoplasm
Wet mount	90 (45%)	Nil	Nil
Pap stained Cytospin smear	120 (100%)	120 (100%)	120 (100%)

Table 22: Nuclear Characterstics Of Neutrophils In Wet Mount And Pap Stained Cytospin Preparation(n=120).

Nuclear characteristics	Smudgy chromatin	Moderately crisp chromatin	Crisp Chromatin
Wet mount	100 (83.3%)	20 (16%)	Nil
Pap stained Cytospin smear	Nil	80 (66%)	40 (33%)

Epithelial Cells In Urine Sediment

On microscopic examination of urine sediment based on morphology epithelial cells were further categorised in to squamous epithelial cells, renal tubular epithelial cells and transitional (urothelial) cells.

Squamous Epithelial Cells (SEC) In Urine Sediment

Wet Mount Preparation

Out of 200 urine samples, sediment from182 (91%) samples showed presence of squamous epithelial cells on wet mount preparation.
The number of squamous epithelial cells/HPF varied from <5 to >20.

The squamous epithelial cells were largest epithelial cell observed on wet mount preparation of urine. They were polygonal in shape with central nucleus and indistinct cell borders arranged singly or in clusters. In 82 samples from females they were in small to large clusters. The nuclear details were not clearly made out.

Cytospin Preparation

182 samples with presence of squamous epithelial cells on wet mount preparation were observed after Cytospin preparation. Number of squamous epithelial cells/HPF varied from >5 to >20.

The cells were in small to large clusters, cytoplasmic and nuclear details were clearly observed. There was differential staining of cytoplasm, indicating the stages of maturity of squamous cells, parabasal cells, superficial and intermediate cells were easily identified. Morphological details of nucleus were clear. Folding of the cytoplasm of intermediate cells and variation of nuclear size and metaplastic squamous cells were better identified.

Correlation Of Number Of Squamous Epithelial Cell – Wet Mount And Cytospin Smear

- 30 samples of urine showed with 1-5SECs/HPF on wet mount showed 6-10SECs/HPF on Pap stained Cytospin smears.
- 66 samples of urine with 6-10 SEC/HPF on wet mount showed 11-20 SECs/HPF on Pap stained Cytospin smears.
- 47 samples of urine showed 11-20 SEC/HPF on wet mount showed >20 SECs/HPF on Pap stained Cytospin smears.
- 19 samples of urine showed >20SECs/HPF both on wet mount and Pap stained Cytospin smears.

Correlation Of Number Of RTE cells/HPF- Wet Mount And Cytospin Smear

- 04 samples of urine showed 1-5 RTE cells/HPF both on wet mount and Pap stained Cytospin smear.
- 60 samples of urine with 1-5 RTE cells/HPF on wet mount showed 6-10 RTE cells/HPF on Pap stained Cytospin smear.
- 26 samples of urine with 6-10 RTE cells/HPF on wet mount showed 11-20 RTE cells/hpf on Pap stained Cytospin smear.
- 20 samples of urine showed 11-20 RTE cells/HPF on wet mount showed >20 RTE cells/HPF Pap stained Cytospin smears.

07 samples of urine showed >20 RTE cells/HPF both on wet mount and Pap stained Cytospin smears.

Correlation Of Number Of Transitional cells/HPF – Wet Mount And Cytospin Smears.

- 30 samples of urine with 1-5 transitional cells/HPF on wet mount showed 6-10transitional cells/HPF on Pap stain Cytospin smear.
- 22 samples of urine with 6-10 transitional cells/HPF on wet mount showed 6-10 transitional cells/HPF on Pap stained Cytospin smear
- 10 samples of urine with 11-20 transitional cells/HPF on wet mount showed >20 transitional cells/HPF on Pap stained Cytospin smear.

6 samples of urine showed >20 transitional cells/HPF both on wet mount and Pap stained Cytospin smears.

DISCUSSION

Urine examination still remains as a simple investigation for the assessment of renal damage. There is a need for conducting proper urine sediment examination prior to more expensive laboratory investigation. Urine sediment examination is cost effective, simple and yields a lot of valuable information regarding the functional status of the kidney.[9]

Urine sediment examination is of high diagnostic value in patients with high grade carcinoma of the urinary bladder. [10]

Sex Distribution

Of the 200 urine sediments studied, more samples were from males with a M: F ratio of 1.2:1. This is insignificant since non-probability sampling method was used.
M: F ratio of patients included in other studies also shows a slight male preponderance.

Table 43: Sex Distribution- Comparative Studies

Various studies	M:F
Bhagyalaxmi *et al* (2014)[11]	1.4:1
Saimary AL *et al* (2006)12	1.2:1
Ron wald *et al* (2009)[09]	1.5:1
Renshaw A *et al*(2000)[13]	1.7:1
AK Mambatta *et al* (2015)[08]	1.3:1
Present study	1.2:1

Age

Mean age of the patients was 47.21 years with more samples (39%) belonging to patients in 3rd and 4th decade.

RBC

Morphology of RBCs was better appreciated in wet mount compared

to Cytospin preparation. There were no dysmorphic RBCs. RBCs appeared as round or biconcave cells with smooth surface. The increased number of RBCs in Cytospin, showed overlapping and appeared like hemorrhagic areas.

Apland T *et al* (2001) compared wet mount preparation i.e. bright field microscopy with automated urine flowmetry for their ability to differentiate between glomerular and non-glomerular hematuria. They concluded that results of urine microscopy by skilled observer was not different from flow cytometry.[14]

Saimary AL *et al* (2006) conducted a study to determine the role and significance of cytological and microscopic examination of urine in the diagnosis of urinary tract infection (UTI). Wet mount preparation in their study revealed significant presence of RBCs i.e.39% in positive cases of UTI.[15]

Neutrophils

The presence of up to 5 WBCs/HPF is considered normal. Neutrophils are the most common of the WBCs present in urine sediment and have clinical significance hence their identification is important. Both the terms leukocyte and WBC usually refers to neutrophils.[15]

Morphology of lobes of neutrophils was better appreciated in Pap stained Cytospin preparation compared to wet mount urine examination. On Pap stained Cytospin preparation both the nuclear and cytoplasmic details of neutrophils were well appreciated.

Renal Epithelial Cells

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.

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.

Urinary Casts

Presence of casts usually reflects variation in metabolic pathway activity and types of UTI.[16]

Wet mount preparation of urine showed predominantly granular casts i.e. 58% followed by hyaline casts (28.3%) and waxy casts (13.5%). Morphology of these casts was better with wet preparation than Cytospin smear.

Henneberg JR *et al* (2015) compared three different methods of urine analysis, and reported that for identification of casts, microscopic examination still the preferred method.[17]

Crystals

Crystals most often identified in urine usually do not indicate disease. Urine crystals form when a crystalline compound becomes supersaturated or when compound's solubility properties are altered in the urine. [18]

Microscopic examination of urinary sediment is necessary for accurate identification of the type of crystals.

Morphology of crystals observed in both wet mount and Pap stained Cytospin smear were similar to Henneberg JR *et al studies* (2015).

CONCLUSION

Image 1 : Yellow Coloured Urine

Overall cell yield and preservation of WBCs and epithelial cell morphology was better in Cytospin preparation. While RBC were better appreciated in wet mount preparation. Cytospin preparation helped in appreciating details of malignant cells. Where as cast and crystals were better observed in wet mount urine examination.

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. Hence Cytospin smear examination is advised for routine cytological examination of urine.

Image 2 : Wet mount urine examination of granular cast

Image 3: wet mount of urine shows- calcium oxalate crystals

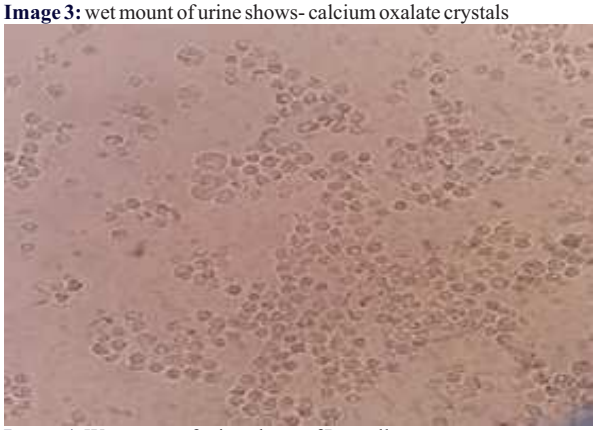

Image 4: Wet mount of urine plenty of Pus cells

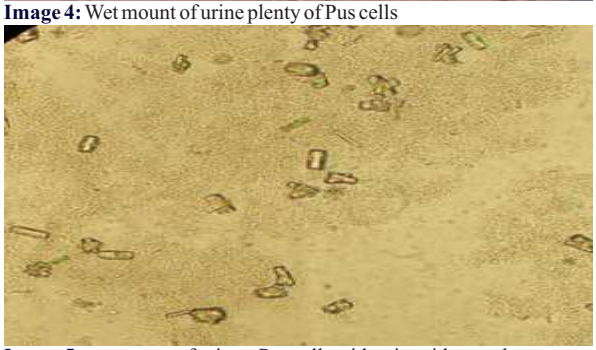

Image 5: wet mount of urine – Pus cells with uric acid crystals

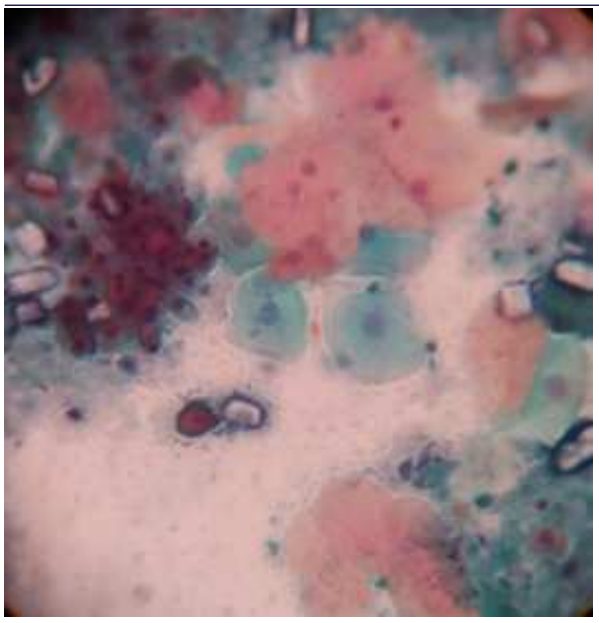

Image 6 : Cytospin Pap smear

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