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Urine microscopy: A mini-review

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Abstract
Urine analysis is a simple, non-invasive and cheap laboratory test that rapidly provides information about functioning of urinary system as well as other organs or systems of the body. In most laboratories microscopic examination of urine is not practice routinely but only performed if physical and chemical urinalysis tests results necessitate microscopic examination. Microscopic examination of urine should be performed on routine basis in all diagnostic laboratories in order to have thorough information on urinary constituents which helps in proper diagnosis of suspected urinary tract and kidney disorders. This article is a mini review on urine microscopy, including the time of sample collection, methods of sample collection, sample preservation, procedure for urine microscopy, a thorough knowledge various organized and unorganized sediments with an emphasis on the significance of microscopic examination of urine to attain correct diagnosis.

Keywords: Urinalysis, microscopy, organized sediment, unorganized sediment

Introduction
About 6,000 years ago, research laboratory on medicine started with the analysis of human urine as *uroscopy*, which is now known as *urinalysis*. The term "uroscopy" derives from two Greek words: "*ouron,"* meaning urine and "*skopeoa,"* meaning to 'behold, contemplate, examine or to inspect'. Earlier physicians considered urine as a window to the internal functioning of the body and mirrored various diseases (Daniel 2022) [2].

Urinalysis means routine examination of urine. Urinalysis tests comprises of physical, chemical and microscopic examination of urine. All these tests have their own significance in detection and estimation of wide range of substances in urine which helps to screen or monitor certain common health conditions, such as kidney disease, diabetes and liver disease, and to diagnose urinary tract infections (UTIs). However, the purpose of urinalysis is to assess the health status of the kidneys. Concurrent serum or plasma biochemical analysis shall maximize the interpretation of urine analysis.

Nowadays, with the advancement of a wide range of tests and techniques like automatic urinalysis, dipsticks methods, reagent strips method etc., microscopic examination of urine equally gains the importance in providing information about functioning of urinary system as well as other organs or systems of the body. Urine microscopy should always be performed as certain abnormalities of urine can only be detected with the help of microscope (Brar *et al.* 2014; Sharique *et al.* 2018) [1, 10]. Presently, urine microscopy is usually performed in various central laboratories without the correct methods, equipment, and professional qualification (Fogazzi *et al.* 1999) [2, 6]. Proper efforts should be made in following the standard protocols for urine collection and handling, knowledge on urinary sediments and capability to organize the findings of urinary sediment into a clinical context. It must be remembered that all kidney related disorders can benefit from the results of urinalysis of good quality, and negative urinary findings also help in the correct evaluation of a renal disorders (Fairley *et al.*1982) [4].

Collection of samples
Urine samples should be collected with minimal contact of the voided urine over the animal's body. Ideally, new/clean containers with tight fitting lids are preferable. Various methods are available for collecting urine sample *viz.* natural method or voided method, manual compression of bladder, catheterization and / or cystocentesis.

Morning urine or freshly voided urine is considered best for microscopic examination of urine sample. It should be analysed within 30 minutes of collection, if not possible then refrigerate the samples or add certain urine preservatives which can preserve the samples for 5-6 hours (Brar *et al.* 2014) [1]. However, refrigeration of samples can cause changes in certain particles *viz*. it can cause precipitation of urates and phosphates giving inaccurate results. In addition to it, preservatives can also change the appearance of particles. Therefore, every emphasis should be given to analyse the samples within 2-4 hours from collection. (NCLLS, 2001; Van der Snoek and Koene 1997) [8, 12].

Procedure for urine microscopy

Centrifuge approximately 5ml of urine sample in a graduated conical tube for 5-10 minutes at 1000-2000 rpm. Discard the supernatant leaving behind the sediment with 1or 2 drops of urine. Then place the sediment mixture on clean glass slide and cover it with a coverslip and observe under microscope. At first, observe under low power (10 X objective) as a scanning process then observe under high power (10 X objective). While examining the urine sediment under microscope emphasis should be given on certain aspects like careful transfer of urine sediment to the slide, use of proper illumination as well as proper magnification, use of clean slides and prevent drying of urinary sediments etc. Urinary sediment can also be obtained without centrifugation *i e* by keeping the samples undisturbed for some time at room temperature to settles down which helps in preventing the loss/lysis caused by centrifugation, (Fogazzi and Garigali 2003) [5] especially for the erythrocyte casts.

Components of urine microscopy

Urinary sediment is broadly categorized into organized sediment or Unorganized sediment.

Organized sediment

Cells

Different types of cells are found in urinary sediment which is listed below. (Figure1.1) [13].

Epithelial cells

Squamous epithelial cells

Flat irregularly shaped cells with a small round nucleus, found singly or in sheets and derived from superficial layer of urethra, vagina or vulva. Squamous epithelial cells are the largest amongst the epithelial cells (Brar *et al.* 2014) [1].

Transitional epithelial cells

Transitional cells are of variable sizes *viz*. round, pear shaped or caudate and derived from the urothelium starting from renal pelvis of the kidneys to urethra (Brar *et al.* 2014) [1].

Renal epithelial cells

Brar *et al.* (2014) [1] reported renal epithelial cells as the smallest epithelial cells that are derived from renal tubules.

Erythrocytes

Spherical in shape, with no internal structure and are smaller than leucocytes. In concentrated urine the RBCs are likely to be crenated while in diluted urine they are swollen and appear as faint colourless rings. Occasional erythrocytes are normally present in the urine. (Brar *et al.* 2014) [1].

Leukocytes or pus cells

Appear as spherical, granular cells that are larger than erythrocytes but smaller than the epithelial cells. Occasional leucocytes are normally present in the urine Increased number of leukocytes in the urine is known as pyuria. If there are 10 or more leukocytes per high power field, it is indicative of pyuria (Brar *et al.* 2014) [1].

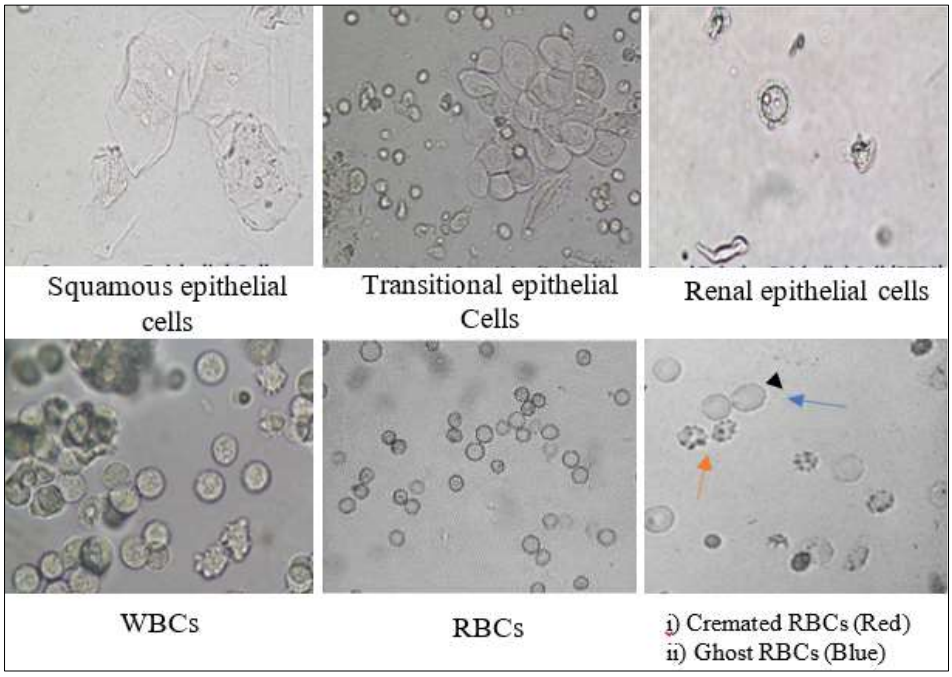

Fig 1: Organized urinary sediment (different types of cells)

Cast

Casts are precipitated proteins appearing as cylindrical bodies and are formed principally in the lumen of the distal tubules and in the collecting tubules of the kidneys (Brar *et al.* 2014) [1]. Different types of casts may be found in urinary sediment which is listed below. (Figure1.2) [13].

Hyaline cast
Appear as homogenous, semi-transparent, colourless, cylindrical bodies having rounded end and mostly found in acidic urine.

Granular cast
These are hyaline casts containing granules which are degenerated epithelial cells and WBCs are incorporated during cast formation. These are more common casts seen in urine.

Epithelial cast
Epithelial casts are formed from the desquamated epithelial cells of renal tubules.

Erythrocyte cast
Casts with embedded margins of erythrocyte membrane may be observed. Appear as homogenously deep yellow to orange in colour and occur due to degeneration of red blood cells.

Leucocyte cast
Leucocyte casts characterized by the presence of many leucocytes adhered to hyaline matrix.

Waxy cast
Waxy casts are opaque, greyish or colorless having dull and waxy appearance which developed from degenerating granular or cellular casts indicating the chronicity or duration of the disease.

Fatty cast
Fatty casts contain fat globules which are highly retractile bodies.

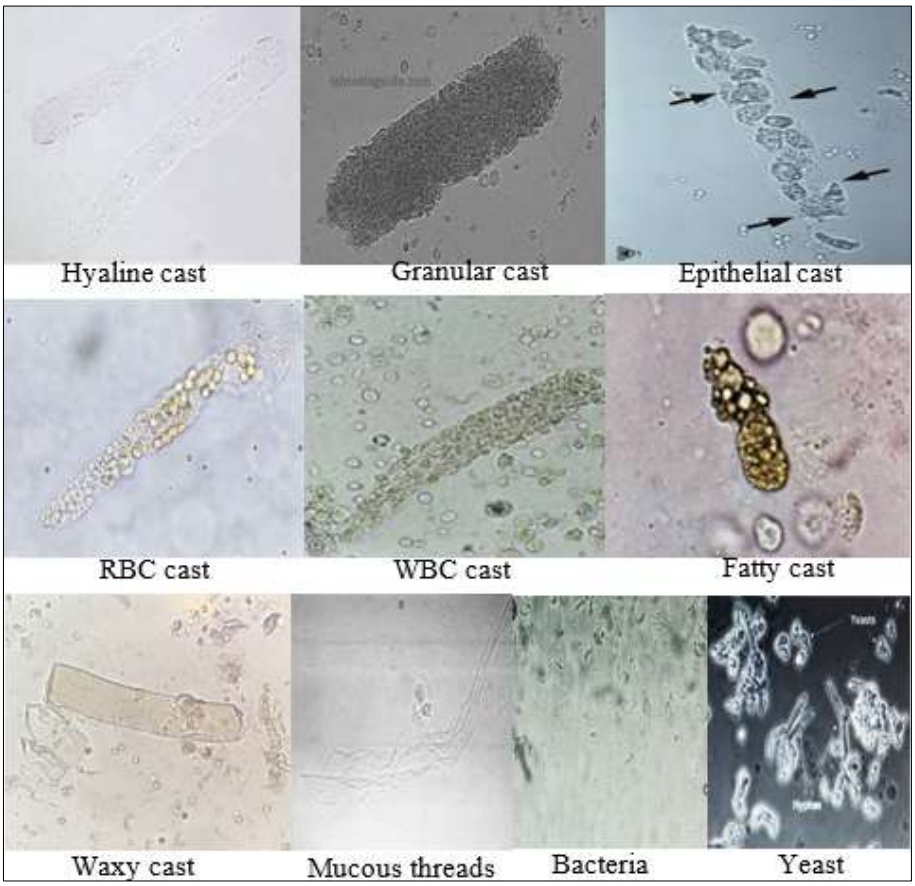

Fig 2: Organized urinary sediment (different types of cast, mucous, microorganism)

Mucus thread
Mucus thread appear as twisted ribbon like structures. Normally, urine of equine contains large amount of homogeneous mucous threads. In other animals' presence of these threads is an indication of contamination from the genital secretions (Brar *et al.* 2014) [1].

Microorganisms
Bacteria: Presence of large numbers of bacteria suggest bacterial infection anywhere in the urinary tract especially when associated with abnormal quantities of WBC and RBC. Bacteria are present in cystitis, pyelonephritis, metritis, vaginitis etc. (Brar *et al.* 2014) [1].

Yeast
Colourless, round to ovoid bodies larger than bacteria, but smaller than leucocytes. Its presence in urine indicates contaminations but yeast infection in the urinary tract of domestic animals is rare (Brar *et al.* 2014) [1].

Fungi
Show distinct hyphae. They are common contaminants. Both yeast and fungi occur as common contaminants or as infection in immunocompromised patients.

Protozoa are rare in urine, may be seen as contaminants of faeces. Trichomonads and Giardia are usually the result of faecal contaminations or contamination by genital secretions.

Parasites

Parasites ova that may be seen in urinary sediment include Ova of *Stephanurus dentatus* (Swine kidney worm), *Dioctophyma renale* (Giant kidney worm of dog), *Capillaria plica* (Bladder worm of dog) and *Dirofilaria immitis* which is a microfilaria that may rarely appear in urine.

Spermatozoa

Sometime spermatozoa may appear in urinary sediment which indicates the urine has been mixed with varying amount of semen.

Unorganized sediment

Fat droplets

Fat droplets are round refractile bodies of varying sizes. It stains orange to red by Sudan dye.

Crystal

Crystals found in urine that is saturated with crystallogenic substances and their presence may be a risk factor for urolithiasis. The presence of crystals in urine depends upon the urine pH, specific gravity, the solubility and concentration of crystalloids, colloids.

Acidic urine may contain amorphous urates, uric acid and less commonly, calcium oxalates and sodium urate. Acidic urine also contains leucine, tyrosine, cystine and rarely hippuric acid. Alkaline urine may contain dicalcium phosphate, triple phosphate, calcium carbonate (especially in the horse), amorphous phosphate and rarely ammonium urate crystals. (Table 1, Figure 2)[14]

Table 1: Crystals found in Urinary sediment

Crystal	Reaction	Colour	Appearance
Uric acid	Acid	Yellow	Rhombic or irregular plates, prisms, rosettes, oval with pointed end
Amorphus urates	Acid	Pink (Grossly) Yellow (Microscopically)	Granules
Calcium oxalate	Acid, neutral or alkaline	Colourless	Octahedral or envelope (small square crossed by 2 intersecting diagonal lines), Dumb bell shaped
Hippuric acid	Acid, neutral or slightly alkaline	Colourless	Prism, Plates or needles
Calcium carbonate	Alkaline	Colourless	Spheres, ovals & dumb bells
Triple phosphate	Alkaline	Colourless	Prisms with oblique ends (coffin lids) feathery
Amorphous phosphates	Alkaline	Colourless	Granules in masses
Ammonium biurates	Alkaline	Yellow	Spheres often covered with spicules, dumb bells or sheaves of needle
Bilirubin	Acidic	Yellow or dark red	Needles, plates or granules
Leucine	Acidic	Yellow	Spheres with radial and concentric striation
Tyrosine	Acidicic	Colourless	Fine needles usually arranged in sheaves with a constriction at middle
Cystine	Acidic	Colourless	Hexagonal plates
Cholesterol	Acidic	Yellow	Overlapping Flakes

Clinical Significance

Urine microscopy is very important and should not be omitted as many abnormalities in a urine sample can only be detected by examining the urinary sediment.

Epithelial cells

Squamous epithelial cells have little diagnostic significance. However, transitional cells are seen in cystitis and pyelonephritis while renal epithelial cells observed in acute interstitial nephritis (Brar *et al.* 2014) [1].

Erythrocytes

Presence of excessive numbers of erythrocyte in urine is called hematuria which may occur in these conditions *viz.* contamination from genital tract, haemorrhage, trauma somewhere in the genitourinary tract, faulty catheterization, bladder puncture and manipulation, urolithiasis, renal infarction, neoplasia, parasites etc. (Brar *et al.* 2014) [1].

Clinically, they show morphological variations, Isomorphic erythrocytes are similar to erythrocytes found in the blood, suggestive of hematuria of urological origin. Dysmorphic erythrocytes have irregular shape and contours and found in glomerular disease. Hematuria is considered to be of glomerular origin when 40% or more are of dysmorphic and 5% or more erythrocytes are acanthocytes (Graham and Galloway 2001) [7].

Leukocytes

Urinary tract infection is the most common cause of pyuria and other condition includes contamination from genital tract *viz.* vulvitis, vaginitis, balanitis metritis etc. and contamination from urinary tract *viz.* urethritis, cystitis, pyelitis, pyelonephritis, nephritis etc. (Brar *et al.* 2014) [1].

Casts

Presence of cast indicates varying degrees of renal changes-renal irritation, renal inflammation and renal degeneration.

Presence of hyaline cast indicates the mild form of renal irritation, febrile condition after anaesthesia, exercise and circulatory disturbances while granular cast presence may indicate a more severe type of renal disease than hyaline cast, for it represents the disintegration of renal tubular epithelium due to nephrotoxins (Ex. aminoglycosides), hemoglobinuria or myoglobinuria induced nephropathy, renal ischemia (Brar *et al.* 2014) [1].

Epithelial cast are seen in acute nephritis whereas presence of erythrocyte cast is an indication of hemorrhage in renal tubule and leucocytic cast are frequently observed in pyelonephritis and kidney abscess (Brar *et al.* 2014) [1]. Some researchers reported the presence of erythrocyte cast in conditions like glomerulonephritis, vasculitis, acute tubular injury/necrosis, tubulointerstitial nephritis; as well as the presence of leucocytic cast in conditions like interstitial nephritis, glomerulonephritis, renal inflammatory processes. (Echeverry

et al. 2010; Patel, 2006; Simerville *et al.* 2005) [3, 9, 11].

Fatty cast are indicative of degenerative tubular disease associated with lipid deposition and diabetes mellitus Whereas presence of waxy cast indicates chronic degeneration of renal tubules and are observed in advanced severe nephritis and amyloid degeneration of kidney (Brar *et al.* 2014) [1].

Microorganisms

Presence of large numbers of bacteria is called bacteuria which may occur in conditions like cystitis, pyelonephritis, metritis, vaginitis etc. (Brar *et al.* 2014) [1].

Yeast are common contaminations in urine samples but yeast infection in the urinary tract of domestic animals is rare and presence of fungi indicates contamination from external environment. Both yeast and fungi occur as common contaminants or as infection in immunocompromised patients (Brar *et al.* 2014) [1].

Fat droplets

Lipuria (presence of fat in urine) is seen under high fat diet, obesity, rupture of lymphatics, diabetes mellitus and hypothyroidism. It commonly occurs in cats due to fatty metamorphosis of renal tubules (Brar *et al.* 2014) [1].

Crystals

The presence of crystals in urine is not always related to pathological conditions or diseases and seldom has clinical significance. However, the presence of crystals in urine may be considered as a risk factor for urolithiasis (Brar *et al.* 2014) [1]. And several types of crystals are related with certain diseases like cholesterol crystals are found in nephrotic syndrome and polycystic renal disease and; leucine and tyrosine crystals are linked with severe liver disorders (Echeverry *et al.* 2010; Simerville *et al.* 2005) [3, 11]. Uric acid crystals are associated with hyperuricosuria and uric acid nephropathy while calcium oxalate crystals are found in ethylene glycol poisoning and hyperoxaluria (Echeverry *et al.* 2010; Simerville *et al.* 2005) [3, 11]. Amorphous Phosphate crystal is observed in urinary sediments in conditions like overactive parathyroid glands, bone metastases, prolonged immobilization, diet with calcium etc. while triple phosphate crystal is associated with urinary tract infections caused by urease-producing bacteria (*Proteus, Klebsiella*). Cysteine crystal is observed in Cystinuria and sulphur crystals is seen after ingestion of antibiotics containing sulfa etc. (Echeverry *et al.* 2010; Simerville *et al.* 2005) [3, 11].

Conclusion

Urine microscopy is a valuable test commonly used in clinical practice. Emphasis should be given on proper method of urine collection as well as on the time of examination; also on keeping records on parameters like food, drugs, exercise, room temperature, daylight, etc in order to avoid possible false-positive or false-negative results. Nowadays, various automated analysers have been developed and are used in diagnostic laboratories to screen a large number of samples within a short period of time. However, these analysers are believed to be insufficient in determining certain constituents of urinary sediment, therefore microscopic examination of urine by a skilled examiner is required to avoid erroneous result in patients with urinary tract infections and renal diseases.

Conflict of Interest

The authors declare no conflict of interest

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