Review Article

Diagnostic Utility of Urine Microscopy in Kidney Diseases

Abstract

Urine sediment analysis is a highly valuable yet underutilized test in today's advanced medical landscape. The analysis of urine sediment is a simple, cost-effective, and powerful diagnostic tool in the hands of a skilled nephrologist, generally in all kidney diseases and particularly more so in the setting of acute kidney injury (AKI). The impact of AKI is far-reaching and encompasses elevated mortality rates, increased morbidity, longer hospital stays, and higher overall healthcare expenses. Timely and compartmental diagnosis of AKI with the use of a simple urine sediment analysis leads to targeted therapeutic strategies and also serves as a prognostic guide. The widespread use of automated analysis in recent times has its own set of limitations, as it fails to identify pathological casts, crystals, and dysmorphic red blood cells (RBCs). Hence, it is the need of the hour to learn this time-honored art of urine sediment analysis, to provide comprehensive patient care.

Keywords: *AKI, kidney diseases, microscopy, urine sediment*

Payal Gaggar[1], Sree B. Raju[2]

[1]Department of Nephrology, Medical Trust Hospital, Kochi, Kerala, [2]Department of Nephrology, NIMS, Hyderabad, Telangana, India

Corrresponding author:
Payal Gaggar, Department of Nephrology, Medical Trust Hospital, Kochi, Kerala, India. E-mail: payalgaggar04@gmail.com

DOI: 10.25259/ijn_362_23

Received: 16-08-2023
Accepted: 03-11-2023
Published: 28-05-2024

Introduction

Rayer and his students in Paris pioneered clinical urine microscopy in the late 1830s. As microscope technology advanced in the mid-1900s, urine sediment analysis became an essential component of clinical examination.[1] Careful analysis of the concentrated particles in urine under a microscope, performed by an experienced nephrologist, plays a vital role in both the identification and treatment of various kidney-related ailments. A skillful and motivated urine sediment examination provides a window into the kidney, and hence has been considered as the "liquid biopsy." Utilizing it as a diagnostic aid proves particularly beneficial when evaluating individuals with kidney diseases in general and acute kidney injury (AKI) in particular. Acute tubular necrosis (ATN) is the leading cause of AKI in hospitalized patients, followed by prerenal azotemia.[2] Since the treatment approaches and outlook for prerenal azotemia and ATN significantly vary, it is crucial to distinguish them promptly based on clinical indicators and laboratory parameters.

In this review, we will discuss the various components of urine sediment [Figure 1] seen in the different etiologies of AKI – prerenal AKI, ATN, acute interstitial nephritis (AIN), nephrotic syndrome, nephritic syndrome, and crystal-induced AKI.

Procedure of urine sediment analysis

For sediment analysis, a second catch sample is preferred. The first void sample is susceptible to lysis of cells and casts [Tables 1 and 2] after prolonged standing of urine in the bladder overnight, hence should be avoided. An alkaline pH and a low osmolality of urine lead to lysis of cells and casts, making interpretation of urine sediment difficult. To prevent material breakdown, the European analysis guidelines recommend examining urinary particles within 1 h after voiding at room temperature or within 4 h if refrigerated. To ensure accurate identification of crystals, a separate nonrefrigerated aliquot is essential, as refrigeration can cause phosphates and urates to precipitate. If the urine sample cannot be analyzed within 4 h of refrigeration, preservatives such as formaldehyde-based solutions, buffered boric acid, and formate-based solutions should be added. However, the presence of preservatives can potentially impact the chemical characteristics and change the appearance of particles. Therefore, it is recommended to analyze the sample

How to cite this article: Gaggar P, Raju SB. Diagnostic Utility of Urine Microscopy in Kidney Diseases. Indian J Nephrol. 2024;34:213–21. doi: 10.25259/ijn_362_23.

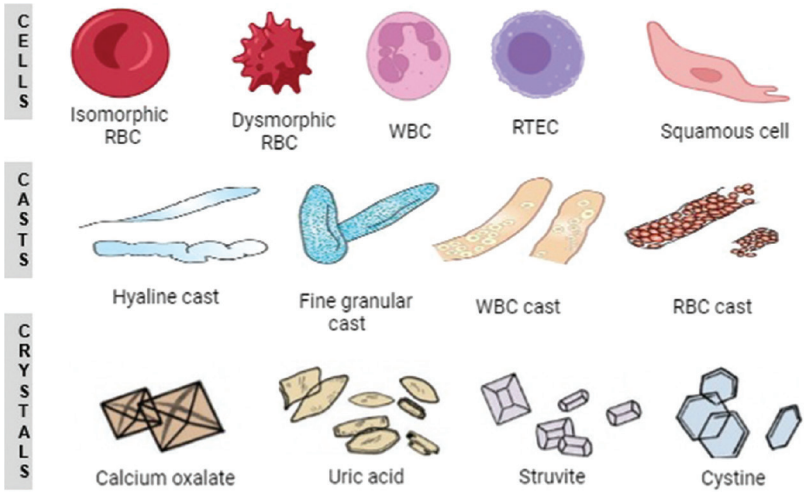

Figure 1: Different types of formed elements seen in the urine sediment analysis. RBC: red blood cell, WBC: white blood cell, RTEC: Renal tubular epithelial cells.

Table 1: Types of cells in urine sediment and their characteristics

Cells	Identification	Remarks
RBCs	Smooth, biconcave discs, 6–8 μm in diameter, no nucleus In hypertonic urine, RBCs shrink and become crenated In hypotonic urine, RBCs swell and lose their hemoglobin, forming ghost cells	Normal- 0–3/hpf or 3–12/μl of urine Isomorphic RBCs → nonglomerular or urological Dysmorphic RBCs → glomerular hematuria
WBCs	Small, regularly round cells with granular cytoplasm with a size of 10 μm with lobed or segmented nuclei (neutrophils) In hypotonic urine, cells swell with Brownian motion of nuclei and cytoplasmic organelles called as the glitter cells	Normal- 0–8/hpf or 10/μl of urine Neutrophils are the predominant type of WBCs seen in urine
RTEC	Round to oval or rectangular, large central or eccentric nucleus, granular cytoplasm with a size of 14 μm Nucleus cytoplasmic ratio- 1:1	Normal- 0–1/hpf Represents damage to tubular epithelium
Transitional (urothelial) cell	Round or ovoid cell; round, centrally placed nucleus with an average size of 30 μm Nucleus to cytoplasmic ratio- 1:2	Normal- 0–1/hpf Represents the cells lining calyces, renal pelvis, ureter, bladder, proximal urethra (in males)
Squamous cell	Large cells of rectangular or polygonal shape with small nuclei and abundant cytoplasm with an average size of 55 μm. Nucleus to cytoplasmic ratio- 1:5	Represents contamination of the urine sample

hpf: high-power field; RBC: red blood cell; RTEC: renal tubular epithelial cell; WBC: white blood cell.

promptly after voiding.[3] About 10 ml of fresh/properly stored urine is centrifuged in a conical tube at 400 g for at least 5 min. By utilizing suction, a quantity of 9.5 ml of supernatant urine is extracted, leaving behind the pellet. The pellet is suspended in the small amount of urine that remains in the tube through gentle agitation. A standardized glass slide is employed, and using a pipette, a single droplet of urine is deposited onto it. Subsequently, a cover slip is placed over the droplet.[4] Examination under bright field microscopy provides better resolution, especially of a stained sample. Dark field microscopy provides lower resolution but allows visualization of objects that are unstained or transparent. It is especially helpful in identification of lipids and crystals as it gives a bright image against a dark background. Phase contrast microscopy is a valuable technique for observing transparent structures such as hyaline casts and particles with a low refractive index. It improves the visibility of the structure's outline and is especially beneficial for visualizing dysmorphic red blood cells (RBCs).[5] Polarized light is useful for identification of lipids, crystals [Table 3],[6] and contaminants such as starch and synthetic fibers. At least a minimum of 10 fields (optimally 20) are examined under both low (100×) and high (400×) magnification. The sediment is observed without the use of staining or can be treated with Sternheimer Malbin (SM) stain to differentiate nucleated cells from other structures. Sudan stain can be used for the lipids. The utilization of Wright/Hansel stain

Table 2: Types of casts in urine sediments and their characteristics

Casts	Identification	Remarks
Acellular casts		
Hyaline casts	Colorless fibrillary matrix composed of Tamm–Horsfall protein, low refractile index	Increased numbers with strenuous exercise, dehydration, fever
Granular casts	Granules can be fine or coarse. Derived from cellular degeneration	Distinguishing finding in acute tubular necrosis, especially if seen with RTEC casts
Waxy casts	Homogenous, broad, waxy-appearing cast with high refractile index. Frequently cracked or indented edges	Seen in patients with renal failure, both acute and chronic
Fatty casts	Contains fat droplets, oval fat body, or cholesterol crystals within the cast matrix Cholesterol globules form Maltese cross under polarized microscopy	Seen in nephrotic range proteinuria
Cellular casts		
RBC casts	RBCs within the matrix	Distinguishing feature of nephritic syndrome, rarely may also be found with AIN
WBC casts	WBCs in the matrix	Can be seen in AIN, nephritic syndrome, pyelonephritis
RTEC casts	RTEC in the matrix	Seen in acute tubular necrosis, AIN
Others		
Pigmented casts	Hemoglobin casts Myoglobin casts Bilirubin casts	Associated with RBC casts Seen in rhabdomyolysis Seen in liver failure
Bacterial casts	Bacteria within matrix	Bacterial pyelonephritis
Crystal casts	Crystals within cast	Seen with renal calculi formation or drug precipitation

RBC: red blood cell; RTEC: renal tubular epithelial cell; WBC: white blood cell; AIN: acute interstitial nephritis.

Table 3: Classification of crystals

Crystal	pH	Birefringence	Appearance
Common crystals			
Uric acid	≤5.8	+	Pleomorphic, diamond, or rhombic; can form layers or rosettes
Amorphous urates	≤5.8	+	Irregular granules
Calcium oxalate	5.4-6.7	mono-	Dihydrate- envelope form
Calcium phosphate	≥7.0	-hydrate)	Monohydrate- dumb bell shaped
Triple phosphate	≥7.0	+	Prism, star-like particles or needles of varying sizes
Amorphous phosphate	≥7.0	+	Coffin lid appearance
Pathological crystals		-	
Cholesterol	5.4–6.7	-	Flat, rectangular plates with notched corners
Cystine	Variable	-	Hexagonal plates, often layered
2, 8-Dihydroxyadenine	≤5.8	+	Spherical brownish crystals with radial striations from the center
Leucine	≤5.8	+	Spheres with concentric circles
Tyrosine	≤5.8	+	Fine delicate needles in clusters
Due to drugs[a]			
Sulfadiazine	≤5.8	+	Shocks of wheat or shells with radial striations
Acyclovir	≤5.8	+	Needles with sharp or blunt ends
Indinavir	≤5.8	+	Irregular plates- crosses/stars like
Ciprofloxacin	≤5.8	+	Needles- stars/fan like
Amoxicillin	≤5.8	+	Shocks of wheat or broom brush like

[a]Other drugs leading to crystal formation include triamterene, primidone, vitamin C, orlistat, and felbamate

for urine eosinophils is no longer recommended, as the presence of urine eosinophils is not considered specific or sensitive enough to diagnose AIN.[7]

Urine sediment examination by different etiologies of AKI

Prerenal AKI

In a study by Mehta *et al*.,[2] prerenal AKI accounted for 17% of AKI cases among hospitalized patients. The urinary sediment typically appears unremarkable or shows the presence of hyaline casts[4] in prerenal AKI.

Acute tubular necrosis

In cases of ATN, shedding of tubular epithelium leads to the appearance of renal tubular epithelial cells (RTECs), RTEC casts, and granular casts in the urine sediment.[8] Dense and brownish/burnt amber granular casts are known as muddy brown granular casts (MBGC). MBGC have 100% specificity and 100% positive predictive value (PPV) for predicting ATN on biopsy.[9] Several scoring systems for urine microscopy have been developed based on the quantification of RTECs, RTEC casts, and granular casts [Table 4]. These scoring systems have excellent specificity for AKI and also correlate well with severity of AKI. The AKI cast scoring index created by Perazella *et al*.[10] showed a PPV of 100% and a negative predictive index (NPV) of 42% for the diagnosis of ATN in hospitalized patients with AKI, when the score was ≥2 (i.e. presence of at least one granular cast or RTECs). These scoring systems are also used to predict a more severe course of AKI. A greater urine sediment score was linked to a higher Acute Kidney Injury Network (AKIN) stage of AKI, and individuals with a urine sediment score of ≥3 had a relative risk of 7.3 for experiencing worsening AKI compared to those with a score of 0.[11] According to a study conducted by Chawla *et al*.,[12] patients with a higher cast scoring index had poorer renal recovery compared to those with a lower index (2.55 ± 0.9 vs 1.7 ± 0.79, $P = 0.04$). The study also showed that the cast scoring index is useful in predicting the nonrenal recovery. Bagshaw *et al*.[13] also concluded that elevated urinary microscopy scores were linked to increased adjustive odds of worsening AKI, and they correlated with initiation of RRT and mortality.

Table 4: Review of urine microscopy scoring systems

Study	Scoring system
Chawla *et al*. (2008)[12]	Grade 1: no casts or RTEC
	Grade 2: at least one cast or RTEC, but <10% of lpf
	Grade 3: many casts or RTECs (10%-90% of lpf)
	Grade 4: sheet of MBGC and RTEC in >90% of lpf
Perazella *et al*. (2010)[10]	0 points: no casts or RTEC seen
	1 point each: one to five casts/lpf or one to five RTECs/hpf
	2 points each: ≥6 casts/lpf or ≥6 RTECs/hpf
Bagshaw *et al*. (2011)[13]	0 points: no casts or RTEC seen
	1 point: one cast/lpf or one RTEC/hpf seen
	2 points each: two to four casts/lpf or two to four RTECs/hpf
	3 points each: ≥5 casts/lpf or ≥5 RTECs/hpf

hpf: high-power field; lpf: low-power field; MBGC: muddy brown granular casts; RTEC: renal tubular epithelial cell.

The presence of MBGC was associated with an increased risk of experiencing a ≥50% increase in serum creatinine from baseline at discharge (acute kidney disease).[9] The drawbacks of urinary microscopy scoring system include limited validation of these scores in large cohorts and the timing of the urinary sediment assessment after the development of AKI, which may affect the predictive value. Varghese *et al.* demonstrated that 20%–24% of patients converted from prerenal AKI to ATN based on the urinary microscopy scoring system, only after a repeat urine sediment analysis was performed ≥2 and ≥4 days of the initial analysis serially. Hence, a serial urine sediment analysis is recommended to improve the yield in patients with nonrecovering AKI of unclear etiology.[14]

Acute interstitial nephritis

AIN encompasses a heterogeneous group of disorders with myriad causes like drug hypersensitivity (e.g. antibiotics, nonsteroidal anti-inflammatory drugs [NSAIDs], anticancer drugs), immunological disorders (e.g. systemic lupus erythematosus, Sjogren's syndrome), infections (bacterial, viral), and idiopathic. AIN is histologically characterized by edema and leukocytic infiltration of the interstitium, and this reflects in the characteristic urine sediment findings of AIN: white blood cells (WBC), RBCs, and WBC casts. Sterile pyuria is seen in 60%–80% of cases of AIN and is more common in antibiotic-induced AIN. However, lack of pyuria does not rule out the possibility of AIN. Eosinophiluria, earlier a sine-qua-non of AIN, is no longer considered sensitive/specific for AIN, as it is also seen in other pathologies like glomerulonephritis, chronic pyelonephritis, prostatitis, renal atheroembolism, and urinary schistosomiasis.[15] RBCs are present in a mean of 50% of cases, making it a nonspecific and insensitive finding for AIN. AIN is also associated with tubulitis and tubular injury, hence RTEC and granular casts are also seen in 86% of cases. RBC casts are generally considered to be associated with glomerular pathology; however, a study conducted by Fogazzi *et al*.[16] revealed presence of RBC casts in 29% of biopsy-proven AIN cases. They proposed that disruption of interstitial blood vessels in AIN could lead to extravasation of RBCs into the tubular lumen, where they mix with uromodulin to form RBC casts. The same study also found WBC casts in only 14% of biopsy-proven AIN, making WBC cast an insensitive finding for diagnosing AIN. Also important to note, 20% of patients with AIN may have a bland urine sediment.

Nephritic syndrome

Nephritic syndrome is characterized by hypertension, proteinuria, active urine sediment, and low glomerular filtration rate. Histologically, acute nephritic syndrome is identified by the presence of intracapillary proliferation, which may or may not be accompanied by the influx of polymorphs or mononuclear cells. In addition, it can manifest as crescent formation (also known as extracapillary proliferation) or glomerular fibrinoid necrosis. This is reflected in the urine sediment analysis as erythrocyturia, leukocyturia, shedding of RTECs, RBC casts, and mixed cellular casts.[7] It was Fairley and Birch who first suggested dysmorphic RBCs to be a hallmark of glomerular bleeding.[17] Dual injury, an initial mechanical damage caused by the passage of RBCs through the glomerular basement membrane followed by osmotic injury during passage through hypotonic tubular fluid, is responsible for dysmorphism of RBCs.[18] Dysmorphic RBCs exhibit irregular central pallor with uneven edges or a distinct target-like appearance. In certain cases, these dysmorphic RBCs may also display small blebs or protrusions. On the other hand, isomorphic RBCs possess a biconcave shape with a central pallor that accounts for approximately half of the RBC diameter. The central pallor displays smooth edges. In urine, these RBCs have the ability to either swell into spherical shapes or shrink into spiked disc. Chu-Su *et al*.[19] documented 28 types of isomorphic and dysmorphic RBCs based on different morphologies. Acanthocytes or G1 cells with membrane protrusions or blebs and doughnut-shaped deformity (loss of cytoplasmic color due to reduced hemoglobin content) are considered to be the most characteristic dysmorphic RBCs seen in glomerular bleeding. The importance of phase contrast microscopy in identifying these dysmorphic RBCs cannot be overemphasized. However, with lack of access to phase contrast microscopy, bright field microscopy with lowering of condenser and SM staining can be used to improve the yield. There is a lack of clear and consistent criteria to definitively distinguish hematuria as either glomerular or nonglomerular in origin. The literature reports varying clinical cut-offs for significant dysmorphic RBCs, ranging from 10% to 90%, based on the study design and the selected population.[20-22] Hamadah *et al*.[23] conducted a study using a dysmorphic RBC threshold of ≥25% and found that it yielded a sensitivity of 20.4% and a specificity of 96.3% for glomerular diseases. In addition, PPV was determined to be 94.6% in this context. Based on these various studies, presence of ≥40% dysmorphic RBCs and/or ≥5% acanthocytes and/or ≥1 RBC cast/50 low-power fields (lpf) is a reasonable cut-off to define glomerular hematuria.[7] Acanthocytes and dysmorphic RBCs serve as relatively specific markers for glomerular damage; however, they exhibit limited sensitivity as isomorphic RBCs are often seen in glomerulonephritis. Isomorphic RBCs may also appear in different nonglomerular situations such as AIN and renal cell carcinoma, as well as in conditions outside the kidneys like nephrolithiasis, urologic cancers, urinary tract infections (UTIs), and excessive anticoagulation. Glomerular injury *per se* without any evidence of UTI may have pyuria and WBC casts. Their presence in association with dysmorphic RBCs/RBC cast is more suggestive of a proliferative glomerulonephritis rather than a nonproliferative glomerulonephritis.[24] Coexistence of tubular damage, in association with acute nephritic syndrome explains the presence of RTECs and RTEC casts in the urine sediment. Besides aiding in the diagnosis, the presence of hematuria, dysmorphic RBCs, and RBC casts is also valuable in assessing the response to treatment and predicting the probability of relapse, particularly in patients with lupus nephritis and pauci-immune vasculitis.[25,26]

Nephrotic syndrome

Nephrotic range proteinuria (≥3.5 g/day), hypoalbuminemia (<2.5 g/dl), and edema are considered the characteristic triad of nephrotic syndrome,[27] which reflects in urine sediment analysis as lipiduria and fatty casts.

Nephrotic syndrome is associated with hyperlipidemia, and lipiduria is thought to be due to ultrafiltration of these lipid fragments, majorly high-density lipoprotein (HDL) particles because of their relatively small size, across the hyperpermeable glomerular basement membrane. Inside the tubules, proximal tubular cells partially reabsorb lipids and transport them to lysosomes for hydrolysis. These lipid-filled tubular cells slough and become oval fat bodies. Within the urinary sediment, lipids can manifest in different forms, such as free-floating fat droplets, or within renal tubular cells or macrophages (oval fat bodies), or within tubular cast (fatty casts), or as cholesterol crystals. Free lipid droplets appear as translucent round particles, either isolated or in aggregates. Under the polarized light, cholesterol and its esters (not other lipids) produce the typical appearance of "Maltese crosses" with symmetrical arms. Lipid droplets could be mistaken for isomorphic RBCs, yeast, round calcium oxalate crystals, or impurities like starch particles (Maltese crosses, with asymmetrical arms under polarized light). Cholesterol crystals are thin, colorless, and transparent plates exhibiting distinct edges and displaying negative birefringence, formed *in vitro*, in refrigerated urine samples.[7] The lipid in the oval fat body and casts can be composed of either cholesterol or neutral fat (triglycerides). Sudan III or IV stains both cholesterol and triglycerides, whereas oil red stains only triglycerides. In the absence of staining, combination of phase contrast microscopy and polarized microscopy is ideal for identification of lipids.[28] Lipiduria is traditionally a marker of nephrotic syndrome, although it may not be invariably present. In a study by Ravigneaux *et al.*,[29] lipiduria was seen in 62.8% of cases with nephrotic syndrome under phase contrast microscopy. They concluded that lipiduria is not predictive of the severity of renal histologic findings. Of note, lipiduria may be seen in non-nephrotic conditions like autosomal dominant polycystic kidney disease

(ADPKD),[30] Fabry's disease,[31] chyluria,[32] lipid emulsion therapy,[33] prostatitis, and oil contamination of the urine specimen.[5]

Crystalluria

Crystals are structures that result from the solidification of urinary solutes in urine. These urinary solutes can consist of a single element, a compound, or a combination of substances, and they are organized in a consistent, repetitive pattern within the crystalline structure. The crystal formation depends on multiple factors like concentration of solute in the urine, the urine pH, and flow of urine through the tubules.[5] For ease of understanding, the crystals can be classified into common crystals, pathological crystals, and crystals due to drugs.[34,35] They can be differentiated on the basis of their structure, urinary pH at which they precipitate, and birefringence under polarizing microscopy, as highlighted in Tables 3 and 5.

Automated systems for urine sediment analysis

Automated analyzers enable the rapid assessment of a large quantity of uncentrifuged samples within a brief timeframe. Frequently used autoanalyzers are IRIS iQ200, Sysmex UF-1000i, Cobas u701, and Sedimax.[4] Automated intelligent microscopy (AIM) encompasses fluids, electro-optics, and image processing. The commonly utilized iQ200 system employs laminar flow technology that aspirates 1 ml of unspun urine and passes 2 µl through a flow chamber, which lies in the object plane of a microscope. In this plane, using stroboscopic illumination, hundreds of images are captured by the charge coupling device (CCD) camera. The camera is equipped with an advanced auto particle recognition software, which uses size, shape, contrast, and texture to identify 12 particle categories of the urine sediment, including cells, casts, crystals, and microorganisms. Due to its multistep nature, manual urine microscopy can be a labor-intensive and time-consuming process involving steps such as centrifugation, slide preparation, and thorough examination of the urine specimen. Lack of methodological standardization and poor interobserver reliability also pose hurdles for proper utilization of manual analysis. Automated urinalysis systems are, therefore, considered time-saving, cost-effective, and standardized systems.

Table 5: Crystals and associated conditions

Crystals	Associated conditions
Common crystals	
Uric acid	Normal subjects, refrigerated urine sample, hyperuricemia secondary to tumor lysis syndrome[34]
Amorphous urates	Normal subjects, refrigerated sample
Calcium oxalate	Normal subjects, primary and secondary hyperoxaluria, ethylene glycol ingestion
Calcium phosphate	Normal subjects and stone formers
Triple phosphate	Normal subjects. Bacterial infections caused by urea-splitting organisms such as *Ureoplasma ureolyticum and Corynebacterium ureolyticum*[35]
Amorphous phosphates	Normal subjects, refrigerated sample
Pathological crystals	
Cholesterol	Nephrotic syndrome, chyluria
Cysteine	Cystinuria – autosomal recessive defect in dibasic amino acid transporter
2,8-Dihydroxyadenine	APRT enzyme deficiency
Leucine	Liver failure
Tyrosine	Inherited disorders of tyrosine metabolism, liver failure

APRT: adenine phosphoribosyl transferase.

However, in the setting of AKI and in patients with kidney diseases, automated systems have limited effectiveness due to their inadequacy to identify sediment particles such as nonhyaline casts, nonsquamous epithelial cells, dysmorphic RBCs, lipids, and crystals in likely pathological samples (partly secondary to the use of an unspun urine).[7] In a limited study conducted at a single center, which included 25 patients diagnosed with ATN, the iQ200 system detected the presence of granular casts in 24% of the samples. Conversely, when the samples were examined by a nephrologist using manual microscopy, granular casts were identified in 72% of the samples ($P < 0.001$).[36] In a different study, automated systems exhibited a strong correlation for erythrocytes ($r = 0.87$, $P = 0.001$) and leukocytes ($r = 0.92$, $P = 0.001$). However, there wasno notable correlation for pathological nonepithelial cells ($r = 0.16$, $P = 0.049$), and the correlation for crystals was very weak ($r = 0.46$, $P = 0.001$).[37]

Urine sediment analysis versus urinary biomarkers in AKI

The last decade has seen a growing interest in developing novel urinary biomarkers to detect AKI early and accurately, as a means to improve the outcomes. The US Food and Drug Administration (FDA)-approved tissue inhibitor of metalloproteinase-2/insulin-like growth factor-binding protein 7 (TIMP-2* IGFBP7) has an area under the receiver operating characteristic curve (AUC) of 0.80 for early identification of AKI and is significantly superior to all previously reported markers of AKI (Kidney injury molecue-1 (KIM-1), Urinary neutrophil gelatinase-associated lipocalin (NGAL), Interleukin-18 (IL-18)).[38] Apart from diagnostic potential, the urinary biomarkers have utility in predicting the progression of AKI and determining necessity for dialysis once AKI is established. Higher urinary IL-18 and NGAL levels after AKI sets in identify individuals with high risk of AKI progression and worse patient outcomes.[39] Elevated levels of urinary Chemokine (C-C motif) ligand 14 (CCL-14) in patients with stage 2/3 AKI following cardiac surgery demonstrated a strong predictive value for the requirement of renal

replacement therapy (RRT) within the next 7 days, with an AUC of 0.91.[40] However, these biomarkers have their own set of limitations. None of the biomarkers reported are completely specific to AKI. They can also be elevated in other conditions such as sepsis, chronic kidney disease (CKD), and UTIs and do not have specific cut-off values that can be universally applied.[41,42] They perform poorly in patients with preexisting CKD and other comorbidities.[43] Currently, there is inadequate evidence to support the notion that early detection of AKI through the use of biomarkers effectively prevents the progression of AKI or proves to be cost-effective.[44]

Studies comparing the urine sediment analysis and urinary biomarkers in AKI have found a good correlation between higher urine microscopy scores and elevated urinary NGAL levels ($P = 0.0003$).[13] Similar predictive value was also found between urine microscopy and urinary KIM-1, IL-18, and urinary NGAL.[45] In terms of reliability in diagnosing AKI, urinary NGAL is more sensitive (sensitivity- 65%, specificity- 65%, AUC- 0.70) and urinalysis with microscopy is more specific (specificity- 91%, sensitivity- 22%, AUC- 0.57) for diagnosing AKI. Like the urinary biomarkers, urine sediment analysis also helps in prognostication. In a study by Perazella *et al*.,[11] higher sediment score was found to be associated with an increasing adjusted relative risk of worsening AKI (adjusted relative risk: 7.3, 95% confidence interval: 4.5 to 9.7 for worsening with a score of ≥ 3 vs. a score of 0).

Quality control

The role of a good internal quality control and external quality assessment to improve the quality and reliability of urine sediment examination cannot be overemphasized. Standardizing the method of preparing the urine sediment is the first step to achieve this. Avoiding delays in investigation after micturition, standardization of centrifugation time and speed, avoiding losses during centrifugation, use of suction rather than decanting to remove the supernatant, gentle resuspension of the pellet to avoid lysis of any casts, and adequate review of the full coverslip area are some of the steps that need strict adherence to improve the yield.[3] Being cognizant of the potential for errors in these stages should serve as a guiding principle when developing the most precise protocol for routine sediment analysis. The identification of clinically significant urine particles should be internally reviewed and externally evaluated (External Quality Assessment schemes). In a small-scale study conducted at a single center involving 26 patients, the accuracy of urine sediment interpretation was compared between laboratory personnel and a nephrologist. The findings revealed that the nephrologist, utilizing manual microscopy, detected a higher number of RTECs, granular casts, and dysmorphic RBCs compared to the interpretations made by laboratory personnel. Even when blinded to clinical history, the nephrologist conducting urinary microscopy achieved accurate diagnoses in over 90% of cases. In contrast, when a second nephrologist relied on automated urinalysis and laboratory-based microscopy report, the correct diagnosis was made only 19% of the time.[46] In a study conducted by Palsson *et al*.,[47] the consistency among nephrologists in identifying various urine sediment findings exhibited mostly moderate to substantial interobserver reliability, but the level of agreement varied substantially. On the other hand, some findings like WBC casts and RTECs showed only fair agreement among the observers. The above studies highlight the importance of manual urine sediment examination and the crucial role of nephrologists in the analysis, advocating the need for sufficient training in this vital cost-effective procedure.

Limitations of urine microscopy

Although it is cost-effective and capable of offering valuable diagnostic insights, urine microscopy analysis still comes with its own array of constraints. It is essential to stress the importance of standardizing procedures, conducting prompt examinations, following sample collection to prevent artifacts and crystallization and addressing interobserver differences, which necessitates the significance of incorporating proper training into the curriculum. In addition, the lack of universal access to phase contrast microscopy and special stains poses obstacles to the precise identification of certain sediment particles. Although urine sediment scores have been used to predict the severity and prognosis of AKI, there is limited validation of these scores in larger cohorts.[48] On occasion, urine sediment may appear unremarkable even when various underlying kidney conditions, such as AIN, proliferative lupus glomerulonephritis, acute tubular injury, and thrombotic microangiopathy, are present.[4] Furthermore, the cells and crystals observed in urine may not necessarily indicate the underlying cause of the disease. Notable examples include uric acid, calcium oxalate, and drug-related crystals found in the urine of asymptomatic individuals. Hence, the importance of a physician's judgment based on the clinical scenario and pretest probability for a likely diagnosis cannot be overemphasized.

Conclusion

Once an inclusive part of nephrology curriculum, the practice of urine microscopy involving the examination of urine sediment is gradually waning and becoming a dying art for nephrologists. But with the ease of performing, cost-effectiveness, and the ability to make diagnostic decisions leading to timely intervention, especially in the context of AKI, urine examination is here to stay. The importance of this art cannot be overemphasized in the current scenario, with the infiltration of nephrologists to second- and third-tier cities which lack trained and equipped central laboratory services at their disposal. So, it is time for nephrologists to go back to spinning the urine,

to add a new dimension to the comprehensive patient care that every patient deserves.

Conflicts of interest

There are no conflicts of interest.

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About the Cover Image

A 39-year-old female underwent an ABO-compatible living related donor kidney transplant. She did not receive any induction. On day 3, the nadir serum creatinine was 1.2 mg/dL. The urine output dropped from day 5, and the serum creatinine inched up to 3.8 mg/dL on day 7. Doppler showed a high resistive index. The Serum Tac level was within therapeutic range.

The graft biopsy shows seven viable glomeruli. The majority of them possess fibrin thrombi in the capillary lumen and also in the feeding afferent arteriole. A moderate degree of acute tubular injury is noted. Peritubular capillaritis (ptc1) is seen. There is no tubulitis or interstitial inflammation (PAS and Masson's Trichrome, 40x). Two arteries included in the specimen did not show endarteritis. Immunohistochemistry showed uninterrupted linear staining for C4d along the peritubular capillaries (20x).

Contributed by Dr. Mahesha Vankalakunti,
Consultant Pathologist, Manipal Hospitals,
Bengaluru