Microscopic examination of urine

Presenter: Dr. Shikha Agarwal
• A/K as ‘liquid biopsy of the urinary tract’.

• Urine consists of various microscopic, insoluble, solid elements in suspension.

• These elements divided into organized and unorganized substances.

• Organized substances include RBCs, WBCs, epithelial cells, cast, bacteria & parasites.

• Unorganized substances are crystalline and amorphous material.

• Main aim of microscopic examination of urine is to identify different cellular elements and casts.
Specimen:

- Cellular elements are best preserved in hypertonic acidic urine.
- First morning, mid-stream, freshly voided concentrated sample is preferred.
- Should be examined within 1-2 hrs. of voiding because cells and casts begin to lyse within 2 hrs of collection.
- If delay is suspected: Formalin (40%) or thymol can be used as preservative; or urine can be refrigerated at 2-8°C (not >1 hr).
- **Method:** well-mixed sample of urine (10ml) is centrifuged for 5 min @ 2000 rpm.
- Supernatant is separated & used for chemical analysis.
- The tube is tapped at the bottom to resuspend the sediment in 1ml of urine.
- A drop of it placed over slide, covered with coverslip and examined as early as possible.
Methods of examining urinary sediment

1. **Brightfield microscopy:**
   - Although brightfield microscopy can be performed to a limited extent on unstained urine preparation, identification of leukocytes, epithelial cells, and cellular casts is difficult.
   - Subdued light is more effective in delineating the more translucent structures of urine such as hyaline casts, crystals and mucous threads.
   - Crystal violet safranin stain is commonly used to increase delineation of the formed elements. Others stain that can be used are 2% soln of methylene blue and toluidine blue.

2. **Phase contrast microscopy:**
   - It is useful for the detection of more translucent formed elements, notably cast that may escape detection using ordinary brightfield microscopy.
   - It helps in hardening the outlines.
Fig: Hyaline casts: A. Brightfield B. Phase contrast microscopy
3. **Polarized microscopy:**

- Used to distinguish crystals and fibers from cellular and protein casts material.
- Lipid droplets or spherocrystals containing cholesterol esters are anisotropic in polarized light, show up brightly against a dark field, and form maltese crosses with crossed polars.
- Crystals, hair and clothing fibers also show up brightly, but do not exhibit Maltese cross forms.

4. **Quantitative counts:**

- Hemocytometer is used for quantifying the elements of urinary sediment.
- The cells and casts from undiluted well-mixed urine are counted and reported as the numbers of cell per microliter.
Elements of urinary sediment

Urinary sediment

- Cells
  - Red blood cells, white blood cells, epithelial cells, oval fat bodies
  - Cellular
    - Red blood cells, white blood cells, renal epithelial cell
  - Noncellular
    - Hyaline, granular, waxy, fatty

- Casts
  - Normal
    - Uric acid, calcium oxalate, amorphous urates, calcium carbonate, phosphates, urate
  - Abnormal
    - Cysteine, cholesterol, bilirubin, leucine, tyrosine, sulfonamide

- Crystals

- Organisms
  - Baceria, yeast cells, T. vaginalis, microfilaria S. haematobium

- Other
  - Sperm

Broad
CELLS IN THE URINE
RBCs

• Appearance: Under high power
  ✓ Unstained normal erythrocytes appear as pale biconcave disks about 7 μm in D.
  ✓ If the specimen is not fresh it may appear as faint, colorless circles or shadow cells because the Hb may dissolves out.
  ✓ In hypertonic urine become crenated and appear as small rough cells with crinkled edges.
  ✓ In dilute urine the cells will swell and rapidly lyse, releasing Hb and leaving only cell membranes (ghost cells).
  ✓ Dysmorphic (cellular protrusion and fragmentation) in GN.

• RBCs may be confused with oil droplets and yeast cells. Oil droplets, however exhibit a greater variation in size and are highly refractile.
  Yeast cells are more oval, show budding. If identification is difficult, 2 preparation may be made and a few drops of acetic acid added to the one. RBCs are lysed in the acidified preparation.

• 0-2 /HPF: Normal
  ≥3/HPF in two of the three properly collected urine sample: Microscopic hematuria.

• Increased no. are seen in GN, LN, Berger’s disease, HSP etc
Fig: RBCs; A. Ghosts cells, B. Fresh, C. Crenated
WBCs (pus cells)

- Neutrophil is the most predominant lymphocyte that appear in urine. Under high power these cells appear as granular spheres about 12 µm in D with multilobated nuclei. Nuclear segments may sometimes appear as small, round discrete nuclei.

- When cellular degeneration has begun, nuclear details may be lost, and neutrophils may then become difficult to distinguish from renal tubular epithelial cells.

- Dilute acetic acid/ supravital stain may enhance nuclear detail so that differentiation may still be possible. Peroxidase cytochemical reaction is also useful in distinguishing neutrophils from tubular cells.

- Ultimately with continued degeneration, neutrophilic nuclear segments fuse, making difficult/impossible to distinguish from mononuclear cells.

- In dilute or hypotonic urine the neutrophils are called glitter cells.

- Normal: 1-2 WBCs/HPF.
  - Pyuria: increase no of WBCs
  - UTI: many white cells in clumps or pus cells ≥10/hpf

- Increased no. are seen in fever, lower UTI, PN, TIN, RTR etc.
Fig: Neutrophils; A. usual appearance. B. with acetic acid
Epithelial cells:

• Any site in genitourinary tract from PCT up to the urethra or from the vagina.
• Normally a few cells from these sites can be found.
• A marked increase indicates inflammation of that portion of urinary tract from which the cell is derived.
• TYPES:-
  1. Squamous epithelial cells.
  2. Transitional epithelial cells.
  3. Renal tubular epithelial cells.
Squamous epithelial cells:

• Most frequent epithelial cells in urine but least significant.
• Lines the distal 1/3rd urethra and vagina.
• Large, flattened cells with abundant cytoplasm and small central round nuclei.
• Cell margins are often folded/rolled.
• Increased no. indicate contamination of urine with vaginal fluid.
Transitional epithelial cells:

• Lines the renal pelvis, ureters, urinary bladder, and upper urethra.
• Smaller than squamous cells, round or pear shaped with a round centrally located nucleus.
• When stained, they have dark blue nuclei with variable amount of pale blue cytoplasm.
• Large clumps or sheets of these cells are seen after catheterization, and in transitional cell carcinoma.
Fig: Transitional epithelial cells with WBCs

Fig: Transitional epithelial cells
a. flattened from superficial layer;
b. deeper cells
Renal tubular epithelial cells:

• Most significant type of epithelial cells found in urine, because their increased no. indicates tubular damage.

• Papanicolaou stain is useful in distinguishing renal tubular cells from other monocular cells in urine.

• Epithelial cells from PCT & DCT occur singly and are large with coarsely granular eosinophilic cytoplasm.

• Epithelial cells from CDs are 12-20 μm in D and are identified by their characteristic cuboidal or polygonal shape and large eccentric nucleus.

• Increased in ATN, PN, viral infection of the kidney, allograft rejection, salicylate or heavy metal poisoning etc.
Oval fat bodies

• Tubular cells that have absorbed lipoproteins with cholesterol and triglycerides leaked from nephrotic glomeruli.
• Under polarized light they show Maltese cross pattern.
• Seen in lipiduria e.g. nephrotic syndrome.

Fig: Polarised anisotrophic fat droplets
Sperm

- Spermatozoa may be present in the urine in the following condition:
  a) Urine of men after convulsions.
  b) Nocturnal emissions.
  c) Retrograde ejaculation.
  d) In urine of both sexes after coitus.
CASTS IN THE URINE
• Cylindrical, cigar-shaped structures that form in distal tubules and collecting ducts and take shape & diameter of lumen, have parallel sides and rounded ends.

• Always renal in origin and indicates intrinsic renal disease.

• Best seen under low power objective (10X).

• All casts are basically composed of precipitate of glycoprotein (Tamm-Horsfall protein) that is secreted by the thick part of the ascending loop of Henle (and possibly the distal tubule).

• Various types of casts are formed when different elements get deposited on the hyaline material (Tamm-Horsfall protein).

• **Hyaline and granular cast** may appear in normal as well as diseased states while all other casts are found in kidney disease.
Fig: Genesis of casts in urine.

1. Stasis, low pH and high salt concentration of filtrate in renal tubules
2. Denaturation and precipitation of Tamm-Horsfall protein
3. Hyaline casts
4. Entrapment of cellular elements in hyaline matrix to form cellular casts
5. Degeneration of cells within cellular casts to form coarse granular casts
6. Prolonged stay of casts in tubules with further degeneration of cells to form waxy casts
7. Broad casts
Hyaline cast

- Most common type of casts in the urine composed of Tamm-Horsfall protein.
- Homogenous, colorless, translucent with brightfield microscopy; pink with supravital staining; and are more easily visualized with phase contrast microscopy.
- 0-2/LPF is normal.
- Increased no. are seen with renal diseases and transiently with exercise, heat exposure, dehydration, heat exposure etc.
Granular casts

- Granules may be large or small and may originate from plasma protein aggregates that pass into the tubules from damaged glomeruli, as well as from cellular remnants of WBCs, RBCs or damaged renal tubular cells.

- Increased no. are seen with glomerular and tubular diseases, tubulointerstitial disease, allograft rejection, renal papillary necrosis, following strenuous exercise.
Waxy cast (renal failure cast)

- With chronic renal diseases, some casts become denser in appearance and are called waxy.
- Differ from hyaline cast in that they are easily visualized because of their high refractive index.
- Under brightfield microscopy they are homogenously smooth with sharp margins, blunted ends and cracks frequently seen along the lateral margin.
- Seen in chronic renal failure, acute and chronic renal allograft rejection.
Fatty cast

- Form when highly refractile fat globules (triglycerides and cholesterol esters) are get embedded in Tamm-Horsfall protein matrix.
- Seen in nephrotic syndrome.

Broad casts

- Diameter is 2-6 times bigger than that of normal casts.
- Indicate tubular dilatation and/or stasis in the distal collecting duct.
- All types of casts may occur in broad forms and are typically seen in CRF.
- Both waxy and broad casts are associated with poor prognosis.
# Cellular casts

Casts to be called cellular should contain at least 3 cells in the matrix.

<table>
<thead>
<tr>
<th>RBC cast</th>
<th>WBC cast</th>
<th>Tubular epithelial cell cast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formed when RBCs get embedded in the THP matrix.</td>
<td>Form when WBCs get embedded in THP matrix.</td>
<td>Formed when tubular epithelial cells get embedded in the THP matrix.</td>
</tr>
<tr>
<td>Brown in colour due to Hb pigmentation</td>
<td>No specific colour</td>
<td>No specific colour</td>
</tr>
<tr>
<td><strong>Have greater diagnostic importance</strong> than any other casts. Help to differentiate hematuria due to glomerular diseases from hematuria due to other causes. RBC casts usually indicate glomerular pathology e.g. GN.</td>
<td>WBC enter the tubules from interstitium and therefore presence of leukocyte indicates tubulointerstitial disease e.g. pyelonephritis.</td>
<td>Seen in ATN, PN, viral infection of kidney, allograft rejection, salicylate or heavy metal poisoning etc.</td>
</tr>
</tbody>
</table>
Renal tubular epithelial cell casts is quiet difficult to distinguish from leukocyte casts, particularly in unstained preparations viewed with brightfield microscopy. Supravital staining, phase contrast microscopy, and Papanicolaou staining are helpful in delineating between these 2 cast. The most distinguishing characteristic of renal tubular cells is their single round nuclei.
CRYSTALS IN THE URINE

• Crystals are refractile structures with a definite geometric shape.
• Usually not found in fresh urine but appear when urine strands for a while.
• Two main types:
  1. Normal (seen in normal urinary sediment)
  2. Abnormal (seen in disease states).
• Crystals found in normal urine can also be seen in some diseases in increased numbers.
• Crystals are identified by their appearance and their solubility characteristics.
Uric acid crystals

- Variable in shape (diamond, rosette, plates). Soluble in alkali and insoluble in acid.
- Increase in
  a) Gout.
  b) Acute leukemia.
  c) High purine metabolism state.
Calcium oxalate crystals

- Colourless, refractile and envelope shaped (other forms: dumbbell, peanut) crystal.
- Can be present in normal urine after ingestion of various oxalate rich foods.
- Increased no. in fresh urine suggest oxalate stone.
- Large no. are seen in ethylene glycol poisoning.
Amorphous urates

• Urates salts of Na, K, Mg, and Ca.
• Usually yellow fine granules in compact masses.
• No clinical significance.

Hippuric acid crystals

• Elongated prism like.
• Rarely seen in urine.
• No clinical significance.
Normal crystals in alkaline urine

Calcium carbonate

- Small and colorless with dumbbell or spherical shapes.
- They may form pairs or clumps. Distinguished from other crystals/amorphous material by their production of CO$_2$ in the presence of acetic acid.
- No clinical significance.
Triple phosphate crystals

- Appear as colorless, 3-6 sided prisms (coffin lid appearance) and occasionally fern leaf.
- Frequently found in normal urine.
- Presence in fresh urine indicates stones in the kidney, staghorn calculi of the pelvis.
Amorphous phosphates

- Granular particles with no definite shape, often dispersed.
- No clinical significance.

Ammonium biurate crystals

Like the typical urate crystals, the have a yellow-brown colour and appear as spheres with radial or concentric striations and irregular projection or thorns, referred to as “thorn apples”.

Amorphous phosphates

Amorphous biurate
Abnormal crystals

Cystine crystals

• Colorless, refractile, hexagonal plates.
• Frequently have layered or laminated appearance.
• Soluble in NH$_3$, 30% HCl and detected by cyanide-nitroprusside test.
• Occur in the urine of patient with aminoaciduria.

Cholesterol crystals

• Flat, rectangular plates with notched (missing) corners.
• Seen in lipiduria e.g. nephrotic syndrome and hypercholesterolemia.
Leucine crystals

• Highly refractile, yellow or brown spheres with radical and concentric striations.

• Found in MSUD, along with tyrosine in severe liver diseases (cirrhosis).
**Tyrosine crystals**

- Colorless or yellow, fine silky needles in sheaves or clumps.
- Seen in liver disease and tyrosinemia (an inborn error of metabolism).
- Dissolve in alkali.

**Sulfonamide crystals**

- Yellow-brown sheaves of wheat with central bindings, striated sheaves with eccentric bindings, round forms with radial striations etc.
- Occurs following sulfonamide therapy.
- Soluble in acetone.
## Reporting of urine microscopy:

<table>
<thead>
<tr>
<th>Sediment – Concentration 1:10</th>
<th>Negative</th>
<th>occasional</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>4+</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs/HPF</td>
<td>0</td>
<td>&lt;4</td>
<td>4-8</td>
<td>8-30</td>
<td>&gt;30</td>
<td>Packed field</td>
</tr>
<tr>
<td>WBCs/HPF</td>
<td>0</td>
<td>&lt;5</td>
<td>5-20</td>
<td>20-50</td>
<td>&gt;50</td>
<td>Packed field</td>
</tr>
<tr>
<td>Casts &amp; Abnormal crystals /LPF</td>
<td>0</td>
<td>&lt;1</td>
<td>1-5</td>
<td>5-10</td>
<td>10-30</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>
ORGANISMS IN THE URINE

Bacteria

• In wet preparation bacteria should be reported only when urine is fresh and without contamination.

• Gram staining of sediment smear can be done for identification.

• When accompanied by pus cells, indicates UTI. Presence of only bacteria without pus cells indicate contamination with vaginal or skin flora.

• May occur as rod, chain or cocci.

• Acid-fast bacilli may be seen in urine sediment, but because of the urethral flora may contain non-pathogenic acid-fast organisms, the presence of tuberculosis in urine must be substantiated by culture and/or PCR methodology.
Fungi: Yeast cell (candida)

- Round or oval refractile structure of approximate the same size of RBCs.
- **Budding** is usually seen.
- Presence in urine suggest immunocompromised state, vaginal candidiasis or DM.
Parasites

- Parasites and parasitic ova may be seen in the urinary sediments as a result of fecal or vaginal contamination. When noted, repeated examination should be performed on a fresh, cleanly voided urine sample.
- Although *Trichomonas vaginalis* may be present in the urine as a result of vaginal contamination, urethral or bladder infection can occur. And, when present, the protozoa should be searched for immediately in the wet preparation of the sediment. Motility of the organism is helpful in making identification.
- In patients with schistosomiasis due to *Schistosoma hematobium*, typical ova are shed directly into the urine accompanied by erythrocytes from the urinary bladder.
URINE FOR MALIGNAT CELLS

- Malignant tumour cells exfoliated from the renal pelvis, ureter, bladder wall and urethrae are best identified by using cytologic techniques.
- Myeloma cells are also seen in urine, both with or without apparent renal involvement.
SPECIAL TEST IN URINE
Urobilinogen:

• Normally a small amount of urobilinogen is excreted through urine.
• Shows diurnal variation with highest level in afternoon.
• Causes of increased level are hemolysis & hemorrhage in tissue.
• Reduced level seen in obstructive jaundice & reduction of intestinal bacterial flora.

Ehrlich’s aldehyde test:

• Ehrlich’s reagent (p-dimethylaminobenzaldehyde) reacts with urobilinogen to produce pink colour. Intensity of colour is proportional to amount urobilinogen present in the urine.
• Presence of bilirubin interferes with reaction so it should be removed before proceeding for the test.
• If porphobilinogen is present in urine it gives same type of reaction, so differentiation between two is must. Watson Schwartz test is performed for the differentiation.
Specific test for urobilinogen:

- The test area of the reagent strip is impregnated with 4- methoxybenzene-diazonium-tetrafluoroborate, which couples with urobilinogen in an acid medium to form a red azo dye.
- This test, unlike the Ehrlich reagent–based methods, is specific for urobilinogen.
Hemosiderin:

- Hemosiderin in urine indicates presence of free haemoglobin in plasma.
- When urine is stained with **Prussian blue stain** hemosiderin appears as blue granules, 1-3µm, singly or in groups, in renal tubular epithelial cells, as amorphous sediment, or as blue granules in casts.
- Hemosiderinuria is seen in intravascular hemolysis.
Myoglobin:

- Myoglobin is a protein present in striated muscle (skeletal and cardiac) which binds oxygen.
- Chemical test used for detection of blood or Hb also gives +ve reaction with myoglobin.
- Test: **Ammonium sulfate solubility test** is used for detection of myoglobinuria. Myoglobin is soluble in 80% saturated solution of ammo. Sulfate, while Hb is insoluble and is precipitated.
- A **positive chemical test for blood done on supernatant** indicates myoglobinuria.
- Causes of Myoglobinuria: *Injury to skeletal or cardiac muscle, e.g. crush injury, myocardial infarction, severe electric shock, thermal burns etc.*
Melanin:

• Never found in normal urine but occurs in patients with malignant melanoma, usually when there is metastasis to liver.

• Fresh urine contain colourless precursor melanogen, which on oxidation forms melanin within 24 hrs.

• Test:

  1) **Ferric chloride test:** 5 ml of fresh urine + 1 ml of 10% FeCl₃ prepared in 10% HCl \(\rightarrow\) **black** coloured melanin (oxidised melanogen).

  2) **Nitroprusside test:** 2 ml of fresh urine + 4 drops of freshly prepared aqueous solution of sodium nitroprusside + 2 drops of 10% NaOH \(\rightarrow\) **Red** colour. This red colour may be because of melanin, acetone or creatinine.

    In final solution when we add 2 ml of glacial acetic acid. **Melanin gives blue to black** colour, acetone **purple** and creatinine **amber** colour.
Ascorbic acid

• Because of its reducing properties ascorbic acid may inhibit several reagent strip reaction (i.e., glucose, blood, bilirubin, nitrite and leukocyte esterase).

• Example: microscopic examination of urine sediment shows >2 RBCs/hpf but heme is not detected by the reagent strip method.

• Test:
  1. **Reagent strip test:**
    ✓ Test area of C-Stix reagent strip is impregnated with phosphomolybdates buffered in an acid medium. The phosphomolybdates are reduced by ascorbic acid to molybdenum blue, this test detects 5 mg/dl of ascorbic acid in urine after 10 sec.
    ✓ Gentisic acid and L-dopa may cause false positive results.

  1. **Gas chromatographic/ mass spectrometric** measurement is a more accurate quantitative method.
Phenylketonuria

- Autosomal recessive inherited disorder due to the absence of enzyme phenylalanine hydroxylase.
- Because phenylalanine is not converted to tyrosine, phenylalanine and other normal metabolites accumulate in abnormal amounts.
- Urinary phenylpyruvic acid, phenylacetic acid and phenylalanine are increased.
- Mental retardation is the major clinical findings.
- Test:
  - Phenistix reagent strip test:- the test area contain ferric ammonium sulfate, magnesium sulfate, and cyclohexylsulfamic acid. At 30 secs. following immersion into urine, the colour of the test area is compared with the colour chart provided.
  - Ion exchange high performance liquid chromatography can be used for quantitative confirmatory testing of abnormal specimens.
Tyrosinuria

• Abnormal metabolism of tyrosine either due to defect in fumerylacetoacetate hydrolase and maleylacetoacetate hydrolase, or deficiency of tyrosine aminotransferase.

• A low tyrosine/phenylalanine diet is the mainstay of therapy.

• Test:
  ✓ **Nitrosonaphthol test** is a non-specific screening test. Tyrosine and tyramine form soluble red complexes with nitrosonaphthol.
  ✓ **Chromatography and quantitative serum assay of tyrosine** are confirmatory test.
Alkaptonuria:

- Phenylalanine and tyrosine are metabolized to homogentisic acid, which is then oxidized to maleylacetoacetic acid by the enzyme homogentisic acid oxidase.

- In alkaptonuria, the enzyme homogentisic acid oxidase is deficient, and homogentisic acid is excreted in urine in large quantities. The urine characteristically turns brown-black on standing or with alkaline pH.

- Test:
  - Ferric chloride and silver nitrate tests are the screening tests. A transient, dark blue colour is seen as 2 drops of 10% ferric chloride soln are added to 2 ml of urine containing homogentisic acid (ferric chloride test).
  - Paper or thin layer chromatography and capillary electrophoresis are confirmatory tests.
Maple syrup urine disease:

• MSUD is one of a group of diseases associated with abnormal branched-chain amino acid metabolism.

• The classic type of MSUD, inherited as an autosomal recessive trait, is marked by severe neonatal vomiting, seizures, stupor, irregular respirations, and often hypoglycaemia.

• Leucine, isoleucine, valine, and their corresponding keto acids are elevated in the plasma and excreted in the urine.

• Test:
  ✓ Dinitrophenylhydrazine screening tests detect the α-keto acid in the urine.
  ✓ Gas or thin layer chromatography, or nuclear magnetic resonance spectroscopy of the urine are the confirmatory test.
Cystinuria:

• Defective transport of cystine by the epithelial cells of the renal tubules and gut, is transmitted as an autosomal recessive trait.

• Although large amounts of the dibasic acids ornithine, lysine, and arginine are also excreted in this disease, cystine is the only one that crystallizes out, with stone formation as a clinical manifestation.

• Tests:

  ✓ **M/E of urine** for colourless hexagonal crystals of cystine.

  ✓ **Cyanide-nitroprusside test**: used for qualitative determination of urine cystine. Cystine is reduced to cysteine by sodium cyanide, and free sulfhydryl group then reacts with nitroprusside to give a red-purple colour.
Indirect test for UTI

These are chemical test in the reagent strip format that can detect significant bacteriuria: nitrite test and leukocyte esterase test.

1. **Nitrite test:** Nitrites are not present in normal urine; ingested nitrites are converted into nitrate and excreted in urine. If gram-negative bacteria (E.coli, Salmonella, Proteus, klebsiella) are present in urine, they will reduce the nitrates to nitrites through the action of bacterial enzyme nitrate reductase. Nitrites are then detected in urine by reagent strip test.

2. **Leukocyte esterase test:** It detects esterase enzyme released in urine from granules of leucocytes. Thus the test is positive in pyuria. The test is not sensitive to leucocytes < 5/HPF.
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THANK YOU