AL-Mustaqbal University Collage. Department of Pathological Analysis Technique. Subject: - Advanced laboratory techniques. Lecture-No.5. General Urine Examination-3.



General Urine Examination

*The Microscopic Examination: -

A microscopic examination may or may not be performed as part of a routine urinalysis. It will typically be done when there are abnormal findings on the physical or chemical examination and the results from all will be taken into account for interpretation.

The microscopic exam is performed on urine sediment-urine that has been centrifuged to concentrate the substances in it at the bottom of a tube. The fluid at the top of the tube is then discarded and the drops of fluid remaining are examined under a microscope. Cells, crystals, and other substances are counted and reported either as the number observed "per low power field" (LPF) or "per high power field" (HPF). In addition, some entities, if present, are estimated as "few," "moderate," or "many," such as **epithelial cells, bacteria**, and **crystals**. Cells and other substances that may be seen include the following:

- 1-Red Blood Cells (RBCs)
- **2-**White Blood Cells (WBCs)
- **3-**Epithelial Cells
- 4-Bacteria, Yeast and Parasites
- 5-Trichomonas
- 6-Casts
- 7-Crystals

Introduction: -

Microscopic examination of urine sediment is of great clinical importance and should never be omitted.

The sediment should be examined for: Type, and Amount Examination of the sediment should always be made shortly after collection so that:

- a. Degeneration and lysis of cellular elements will not occur
- b. Bacteria will not proliferate

<u> Procedure: -</u>

- A. Centrifugation
- \Box Shake the urine sample to make the sample homogenous.
- \Box Put (9-11) ml of urine sample into test tube.

 \Box The recommended parameter for the urine centrifugation is 5 minutes at 2000 RPM.

B. Re-suspension

 $\hfill\square$ The re-suspension procedure has to provide the better homogeneous distribution possible.

 \Box An inadequate re-suspension can be the cause of an uneven distribution although, in the presence of mucus, to which elements may adhere, can cause a significant variation in the different field counts.

C. Examination

□ Place a drop of unstained suspension in a glass slide

□ Place the glass slide on the microscope stage. Examine several fields at 10X and 40X magnification

□ Classify and count casts within LPF (Low Power Fields).

□ Switch to 40X magnification and examine for other elements, i.e., WBCs, RBCs, Epithelial cells, yeast, bacteria, Sperm cells, mucous filaments and crystals.

<u>1-Red Blood Cells (RBCs):</u> Normally, a few RBCs are present in urine sediment (0-5 RBCs per high power field, HPF). Blood in the urine is not a normal finding, but it is not uncommon and is not necessarily a cause for alarm. **<u>Hematuria</u>** is a sign or an indicator that prompts a healthcare practitioner to investigate further to try to determine the underlying cause of the blood.



Overview: Theoretically, no red cells should be found, but some find their way into the urine even in very healthy individuals.

Increased red cells in urine above normal level is termed hematuria

Procedure: RBCs is reported semi-quantitatively as number seen per high power field (HPF) and reported as follow; <5, 5-20, 20-100, or >100.

<u>Normal:</u> RBC up to 5/HPF are commonly accepted as normal.

Appearance:

The appearance of red blood cells (RBC) in urine depends largely on the concentration of the specimen and the length of time the red cells have been exposed. Fresh red cells tend to have a red or yellow color and appear as retractile disks Prolonged exposure results in a pale or colorless appearance as hemoglobin may be lost from the cells and the RBC's begin to have a granted appearance specially in concentrated urine (hypertonic urine).



Note: erythrocytes may lyse in very dilute or highly alkaline urine; Lysed red cells appear as very faint "ghosts", or may be virtually invisible. (Red cell ghosts may simulate yeast)

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Note: The presence of dysmorphic RBC's (distorted cell) in urine suggests a glomerular disease such as a **glomerulonephritis**. Dysmorphic RBC's have odd shapes as a consequence of being distorted via passage through the abnormal glomerular structure. **Note:** the presence of increased number of RBCs with casts and proteinuria suggests bleeding from renal origin

Note: growth hematuria suggests bleeding origin in urethra or bladder neck.

Interpretation:

Hematuria: - is the presence increased amount of red blood cells (erythrocytes) in the urine.

Microscopic hematuria

Small amounts of blood, can be seen only on urinalysis or light microscopy), In microscopic hematuria, the urine appears normal.

Macroscopic hematuria (or "frank" or "gross") Hematuria Gross hematuria is suspected because of the presence of red or brown urine.

<u>-Case-Note</u>: Typically, microscopic hematuria indicates damage to the upper urinary tract (kidneys), while visible blood indicates damage to the lower tract (ureters, bladder, or urethra). But this is not always the case

bladder, or urethra). But this is not always the case.



What are the Causes of hematuria?

Renal causes Post-streptococcal glomerulonephritis): It is the commonest cause of hematuria in children above 3 years. History of preceding streptococcal pharyngitis is usually obtained.

Note: The prognosis of post-streptococcai glomerulonephritia is excellent and complete recovery within few weeks occur but microscopic hematuria may remain for several months.

2-White Blood Cells (WBCs): The number of WBCs in urine sediment is normally low (0-5 WBCs per high power field, HPF). WBCs can be a contaminant, such as those from vaginal secretions.

An increased number of WBCs seen in the urine under a microscope and/or positive test for leukocyte esterase may indicate an infection or inflammation somewhere in the urinary tract. If also seen with bacteria (see below), they indicate a likely urinary tract infection.

Urine Pus cells: -

Overview:

Leucocytes usually enter tubular lumen through and between tubular epithelial cells An increase in urinary WBCs is called **<u>pyuria</u>** and indicates the presence of an infection or inflammation in the genitourinary system.

Microscopic examination and chemical testing are used to determine the presence of leukocytes in the urine.

Procedure: -

WBC is reported semi-quantitatively as number seen per high power field (HPF) and reported as follow; <5, 5-20, 20-100, or >100 (Over 100). Normal: - WBC up to 5/HPF are commonly accepted as normal.



Appearance:

These white blood cells in urine have lobed nuclei and retractile cytoplasmic granules.

White Blood Cells (WBC) in unstained urine sediments typically appear as round, granular cells which are 1.5-2.0 times the diameter of RBCs.

Note: WBC in urine is most commonly neutrophils but nuclei tend to become round as neutrophils age in urine.

Note: Like erythrocytes, WBC may lyse in very dilute or highly alkaline urine; WBC cytoplasmic granules released into the urine often resemble cocci bacteria.

Note: to differentiate between neutrophils and RBCs cells a small drop of GAA is added which enhance the nuclear details and lyse the red blood cells.



Interpretation:

Greater numbers of WBCs (pyuria) generally <u>indicate</u> the presence of an inflammatory process somewhere along the course of the urinary tract.

Positive results are clinically significant and indicate: Acute pyelonephritis Cystitis prostatitis urethritis.

Other causes of inflammation must be considered, such as: Kidney stone Urinary tract neoplasm, including renal cancer and bladder cancer. Acute Glomerulonephritis (non-bacterial)

<u>Note:</u> In bladder infections, WBCs tend to be associated with bacteria, epithelial cells, and relatively few RBCs.

Note: WBC clumps suggest renal origin of WBCs and should be reported when present. **Note:** Pus count greater than 30\HPF suggest acute infection and urine culture is recommended Sterile pyuria

Sterile pyuria: - is urine which contains white blood cells (>10 white cells/mm3) while appearing sterile by standard culturing techniques.

Causes A recently (within last 2 weeks) treated urinary tract infection (UTI) with 'fastidious' organism (an organism that grows only in specially fortified artificial

culture media under specific culture conditions), e.g. *Neisseria gonorrhoeae* and **Renal tuberculosis**.

<u>3-Epithelial Cells:</u> Epithelial cells are usually reported as "few," "moderate," or "many" present per low power field (LPF). Normally, in men and women, a few epithelial cells can be found in the urine sediment.

In urinary tract conditions such as infections, inflammation, and malignancies, an increased number of epithelial cells are present. Determining the kinds of cells present may sometimes help to identify certain conditions. For example, epithelial cells containing large amounts of broken-down hemoglobin (called hemosiderin) may indicate that there were red blood cells or hemoglobin in the urine recently, even if there are none now.





Renal Tubular Epithelial Cells

Urine Epithelial cells

Appearance

Urine epithelial cells are of three kinds: Renal tubule epithelial cells, Bladder epithelial cells, and Squamous epithelial cells.

They are large (the largest cells which can be present in normal urine samples), flat cells with irregular borders, a single small nucleus, and abundant cytoplasm.



Interpretation:

Epithelial cells in urine are generally of little specific diagnostic utility because: Old cells lining the urinary tract at any level may continually slough into the urine. Also in the case of voided samples, even cells from the genital tract can appear in the sample.

Increased number of epithelial cells associated with renal disease such as: Acute tubular necrosis Acute glomerulonephritis Pyelonephritis

Note: In cases of <u>acute tubular necrosis</u>, <u>renal tubular epithelial cells</u> containing large **non lipid vacuoles** may be seen, these are referred to as <u>bubble cells</u>. When lipids cross the glomerular membrane, the renal epithelial cells absorb the lipids and become highly refractive. These are called <u>oval fat bodies</u> and seen in cases of nephritic syndrome.

4-Bacteria, Yeast and Parasites: In healthy people, the urinary tract is sterile and, if the urine sample is collected as a "clean-catch" sample, there will be no microbes seen in the urine sediment under the microscope. Special care must be taken during specimen collection, particularly in women, to prevent bacteria that normally live on the skin or in vaginal secretions from contaminating the urine sample.

If microbes are seen, they are usually reported as "few," "moderate," or "many" present per high power field (HPF).

Bacteria from the surrounding skin can enter the urinary tract at the urethra and move up to the bladder, causing a urinary tract infection (UTI)

Asymptomatic bacteriuria: - is a significant number of bacteria in the urine that occurs without any of the usual symptoms (burning during urination or increased frequency of urination).

Causes: -

Asymptomatic bacteriuria occurs in up to 6% of healthy individuals. It affects 18% of people with diabetes (mostly women), and 20% of elderly individuals (more often women than men). The reasons for the lack of symptoms are not well understood.

Note: Most patients with asymptomatic bacteriuria do not need treatment because the bacteria aren't causing any harm. Treatment with antibiotics is recommended during pregnancy, because it significantly reduces symptomatic urinary tract infections, low birth weight, and preterm delivery.

1- Trichomonas vaginalis is a parasite that may be found in the urine of women, or rarely, men.

<u>5-Urine Ova: -</u>

<mark>5-1)</mark> Trichomonas vaginalis: -

If male: urinary bladder, urethra, prostate

If female: vagina, and cervix

Mode of infection

□ Directly through sexual intercourse as parasite can't live outside body for long period

□ Indirectly through using patients articles as under wear clothes Pathology

Female symptoms

Infection is asymptomatic in 15-20% of cases

The main symptoms associated with Trichomonas infection in women include:

- □ dysuria [painful urination]
- □ Painful sexual intercourse
- □ Burning sensation of the vagina

Green/Yellow, frothy vaginal discharge with a strong foul-smelling odor.

□ Trichomonas infection may cause Vaginitis, and Cervicitis.

<u>Note:</u> Symptoms usually appear in women within 5 to 28 days of exposure. Male symptoms Infection is asymptomatic in 50-90% of cases

Symptoms associated with Trichomonas infection in women include:

🗌 dysuria

- □ Mild urethral discharge
- □ Slight burning after urination or ejaculation
- □ Trichomonas infection may cause Urethritis

<mark>5-2) Schistosome haematobium</mark>: -

Diagnosis: -

Clinical diagnosis Clinical signs & symptoms of urinary schistosomiasis include:

- \Box Haematuria is a common finding.
- □ Proteinuria is frequently present.
- \Box Eosinophils can often be found in the urine.
- □ There is usually also a blood eosinophilia.

Urine Examination: -

Examination of urine for detection of egg this help in acute cases when egg can be detected easily in urine.



6-Casts are cylindrical particles: - sometimes found in urine that are formed from coagulated protein released by kidney cells.

They are formed in the long, thin, hollow tubes of the kidneys known as tubules and usually take the shape of the tubule (hence the name). Under the microscope, they often look like the shape of a "hot dog" and in healthy people they appear nearly clear. This type of cast is called a "hyaline" cast. Normally, healthy people may have a few (0–5) hyaline casts per low power field (LPF). After strenuous exercise, more hyaline casts may be detected.

Other types of casts are associated with different kidney diseases, and the type of casts found in the urine may give clues as to which disorder is affecting the kidney. Cellular casts, such as red blood cell and white blood cell casts, indicate a kidney disorder. Some other examples of types of casts include granular casts, fatty casts, and waxy casts.

<u>6-1) Urine Castes: -</u>

Overview:

Urinary casts are cylindrical aggregations of particulate matter that form in the distal nephron, dislodge, and eventually pass into the urine.

Tamm-Horsfall protein (THP), or Tamm-Horsfall mucoprotein, is the most abundant protein in normal urine. normal daily excreted quantity ranges from 25 to 50 mg.

Function

Uromodulin may act as a constitutive inhibitor of calcium crystallization in renal fluids, and may provide defense against urinary tract infections.

Lindner's mechanism of cast formation.

The cast matrix is formed of uromucoprotein fibrils and is built by a mechanism described by Lindner. The mechanism has four steps:

Initiation: - the first Tamm-Horsfall protein fibrils are fixed to the distal tubular walls, forming a porous sponge like lattice. Since the initial cast has large pores, urine and small debris pass through. This stage could correspond to the early hyaline cast.

<u>Growth</u> as time goes, more and more elementary fibrils, and maybe other proteins, are added to the initial structure, making the pore smaller. The declining pores size reduces the urine flow through the structure.

<u>Maturation</u> After complete obstruction of urine flow, the cast matrix is modified by the tubular activity. The maturation period depends on the nephron activity, especially at the blockade region. Proteins, originating from the surrounding tubular cells, are added to the structure. With renal tubular injury, epithelial cells slough into the lumen of the renal tubules and precipitated on the mucoprotein matrix. With time, the epithelial cells degenerate and can no longer be recognized as cells within the hyaline matrix, thus forming coarsely granular, then finely granular, casts. Waxy casts are the final step in the formation of casts and usually indicate chronic tubular disease.

Evacuation

Because of the surrounding cells activity and the hydrostatic pressure, a time comes where the cast loses its adherence to the tubular wall. The casts are then evacuated by the urinary flow.

Normal: They are absent or very few in urine samples

Note: *Casts are quantified for reporting as the number seen per low power field (10x objective) and classified as to type (e.g., hyaline casts).*