Unit- 4 Interpreting Laboratory Tests

LAB TESTS

1. Why?
   - To aid diagnosis
   - To monitor progress
   - To determine correct dosage

2. Relationship to pharmacy
   - Altered dose in renal failure, liver failure, e.g., digoxin.
   - Drugs may affect lab test results, e.g., urine glucose tests.
   - Monitoring serum drug levels, e.g., tobramycin pre and post levels.
   - Monitoring results of treatment, e.g., effect of antibiotic therapy on WBC in bacterial infection

3. "Normal"
   - Statistical normal, e.g., gaussian curve
   - Depends on equipment and method used; thus may vary between different labs. Use the "normal values" table for appropriate lab.
   - Test may be inaccurate, e.g., hemolyzed RBC and potassium level, failure to refrigerate urine specimens, inaccurate timing - drug post levels.
   - Important to interpret for the patient and disease states involved, e.g., calcium level with hypoproteinemia.

TREAT THE PATIENT, NOT THE LAB DATA.

4. Example of orders that might be written when a patient is admitted to hospital:
   - Admit
   - AAT, DAT
   - Vital signs, routine
   - CBC + diff, platelets, morphology
   - PT/PTT, B12, folate, T4
   - Lytes, BUN, CR, Ca, PO5, Mg
   - AST, LDH, amylase, alk phos, bilirubin (T+D)
   - Serum protein electrophoresis
   - Fasting blood glucose, T/G, cholesterol
   - MSU for C&S
   - 24-hour urine collection for protein and creatinine

ELECTROLYTES

1. Three fluid compartments in the body:
   - Intravascular - inside RBC plus in serum
   - Interstitial fluid
   - Intracellular extravascular
   - Usually it is the **serum concentration** that is measured which usually reflects the concentration in the other compartments, but not always.
   - Concentration depends on water present.

2. Most common measurements:
   - Sodium
   - Major extracellular cation
   - Hyponatremia - often due to edema = relative increase in free body water
     Hypernatremia - often due to dehydration

- Potassium
- Major intracellular cation
- Hypokalemia - tied to alkalosis
- body cells - H+ and K+ exchange
- renal - H+ and Na+ exchange, Na+ and K+ exchange
- Hypokalemia plus digitalis toxicity
- Hyperkalemia - renal failure
- Chloride - major anion
- Note relationship to acid-base balance, renal function.
- Kidney set up to conserve body sodium, excrete potassium and H+
- Calcium - 50% plasma protein may result in abnormally low total serum calcium level, but normal unbound calcium fraction

RENAL FUNCTION

1. Serum creatinine and creatinine clearance

- Creatinine - metabolic product of dephosphorylation of creatine phosphate in muscle
- Relatively constant production hourly and daily
- Excreted by glomerular filtration 70-80% plus tubular secretion
- Relatively sensitive indicator of renal function - creatinine clearance usually parallels GF by +/- 10%
- Factors that may affect test:
- Depends on muscle mass - lower in females
- GFR decreases with age
- Inaccurate at low filtration rates because of the relatively high proportion of secreted fraction
- Creatinine clearance:
- Normal 1.5 - 2.0 mL/S
- Requires 24-hour urine collection
- Or estimate from serum creatinine level:

Cockroft-Gault formula: $ClCr = \frac{(140\text{-age}) \times 1.5 \ (\times 0.85 \ \text{♀})}{\text{SCr umol/mL}}$

2. Blood urea nitrogen (BUN)

- Urea - end product of protein metabolism
- Urea is excreted by glomerular filtration plus 40% is reabsorbed.
- Less sensitive index of renal failure because affected by non-renal parameters;
- protein catabolism rate
- dietary protein intake
- hydration
- Clearance most useful in moderate renal failure
- Serum creatinine rises later than BUN

3. Intravenous pyelogram (IVP)

- A radiologic technique: uses contrast material which is secreted by the kidney tubules, then concentrated. Result shows urinary tract outline, revealing obstructions, plus demonstrates ability of kidney to concentrate.

4. Specific gravity

- Ability of kidneys to concentrate is one of the earliest functions lost in renal disease.

URINALYSIS

- Detects renal or non-renal dysfunction.

1. Colour

- Red: Blood, porphyria, phenolphthalein
- Brown: Blood, alkaptonuria, melanin
- Dark orange: Bile, pyridium

2. Protein

- Glomerular membrane normally prevents most large protein molecules from escaping from blood into urine. The small amount that may normally be present in urine is at a low, usually undetectable level.
- Proteinuria indicates dysfunction or specific state: kidney disease, pregnancy, fever, venous congestion, hypertension, multiple myeloma (Bence Jones protein), severe muscle exertion.

3. Glucose

- Normally actively reabsorbed to a maximum threshold (≅ 180 mg/100 mL blood glucose)
- Threshold varies with age, individual.
- Tests use copper reduction or glucose oxidase reactions, which may be affected by concurrent drug administration.
- To diagnose diabetes mellitus, to monitor diabetic control.

4. Sediment

- Normally few cellular elements are excreted.
- Urine sediment examined under microscope.
- RBC
- WBC
- "Casts":
- muco-protein conglomerates, which may contain RBCs, WBCs, or renal epithelial cells
- or may be mostly protein - hyaline casts
- usually cylindrical since they conform to the shape of the renal tubules
- significance: related to proteinuria, stasis in urinary tract

HEMATOLOGY

- **Complete Blood Count (CBC)**
- Measure hemoglobin and hematocrit (anemia), RBC count, WBC count, WBC differential, RBC morphology

1. Hemoglobin (Hgb)

- Index of O2 - carrying capacity of blood
- in anemia, hemorrhage

2. Hematocrit (Hct)

- Packed cell volume = % of whole blood volume that is RBC
- Rapidly performed, indicates RBC count
- in anemia, hemorrhage

3. RBC

- Examine number per cubic mm, size, shape, colour, maturation, content
- Affected by posture, extreme exercise, excitement, age, sex, altitude, dehydration
- MCV - Mean Corpuscular Volume = average RBC size
- MCHC - Mean Corpuscular Hemoglobin Concentration

4. Classification of anemias

- Classify in order to pinpoint etiology of the anemia.
- Classify by:
  - RBC size = by MCV - microcytic, normocytic, macrocytic
  - RBC colour = by MCHC - hypochromic = low hemoglobin

  e.g., microcytic hypochromic anemia - may be due to iron deficiency
  macrocytic normochromic anemia - associated with folic acid deficiency

5. WBC

- defence
- leukocytosis = increased # WBC - suggests invading organism, tissue destruction • WBC count changes with age, stress, exercise, diurnal rhythm
- WBC differential:
  - Calculate % of each of the 5 types of WBC.

- lymphocytosis - commonly due to viral infection
- eosinophilia - associated with allergic conditions, parasites
- immature band neutrophils - appear if prolonged heavy demand for neutrophils results in release of immature cells = "shift to the left" - referring to usual left to right illustration of neutrophil development

6. Reticulocyte count

- Reticulocyte = immature, non-nucleated RBC
- Normal RBC development: nucleated → reticulocyte → non-nucleated mature RBC
- Increased count means increased RBC production, e.g., hemorrhage, hemolysis, recovery from anemia

7. Platelet Count

- Platelets involved in clotting process
- Chemotherapy → bone marrow depression → thrombocytopenia

BLOOD COAGULATION

1. Prothrombin time (PT)

- Tissue thromboplastin + calcium + patient's plasma area combined.
- Indicates defects in Stage III (prothrombin; factors V, VII, X)
- Altered by liver disease, vitamin K disorders, coumadin therapy
- Also affected by heparin therapy
- Used to monitor warfarin anticoagulation - want PT 2-2.5 x control
- Also to diagnose hemorrhagic problems

2. Activated partial thromboplastin time (APTT)

- Combine incomplete thromboplastin reagent (= partial thromboplastin, no factors), + calcium + patient's plasma + activators)
- Sensitive to defects in Stage II, also severe III and IV
- Used to monitor heparin coagulation - want APTT 1½ - 2½ x normal
- Test also affected by warfarin
- Also to diagnose hemorrhagic problems

BLOOD GASES

- Acid-base balance very important: pH outside 6.8-7.8 will not support life.
- Blood pH is determined by the ratio of bicarbonate ion to carbonic acid: $\frac{base}{acid}$

$$pH = pKa + log \frac{HCO_3}{H_2CO_3}$$

$$pH \propto \frac{HCO_3}{H_2CO_3}$$

- HCO_3 concentration regulated by kidney
- H_2CO_3 concentration proportional to partial pressure of carbon dioxide and regulated by lung

1. Total CO_2

- Measures sum of HCO_3, H_2CO_3 and dissolved CO_2
- Mainly HCO_3 → gives the numerator
- Normal value 20-30 mEq/L

2. pCO_2

- Partial pressure of CO_2 ∝ dissolved CO2
- Since most H_2CO_3 is present as dissolved CO_2, this gives the denominator

3. pH

4. Acidosis/Alkalosis

- Classified as to metabolic or respiratory cause

Metabolic	Respiratory
HCO_3 (tCO_2)	H_2CO_3 (pCO_2)
↑ ALKALOSIS	↓
↓ ACIDOSIS	↑

- For uncomplicated uncompensated cases
- Lungs and kidney try to compensate, but this is not always possible.
- Blood gas measurements used to diagnose or to gauge the severity of the disorder.
- Electrolytes and acid-base - close relationship; e.g.,
- hypochloremic alkalosis
- hyperkalemic acidosis
- Anion gap
  - = calculation of unmeasured anions, used to help diagnose types of acidosis, poisoning by salicylates
  - = plasma sodium concentration minus (plasma bicarbonate plus plasma chloride)

LIVER FUNCTION

1. Serum bilirubin

- Hemoglobin broken down by RES to bilirubin ➔ blood stream ➔ liver where it is conjugated with two glucuronide molecules to give bilirubin diglucuronide = conjugated bilirubin. Conjugated bilirubin is excreted in the bile into the duodenum.
- Two tests:
  1. "Direct-acting bilirubin" - conjugated bilirubin is measured
  2. Measures "indirect bilirubin" = unconjugated bilirubin
- Liver cell damage: Increased total Bi, unconj Bi and conj Bi
- Hemolysis of RBC: Increased total Bi, increased unconj Bi, but conj Bi is normal.

2. Urine bilirubin and urobilinogen

- Bile is excreted into the duodenum where conjugated bilirubin is converted by bacteria into urobilinogen. Most urobilinogen is excreted in feces. Some is reabsorbed into the blood, from which it either goes back to the liver to be excreted again into the bile, or is excreted in the urine.
- In complete bile duct obstruction: No urobilinogen is formed. Stool normally gets its colour from urobilinogen, ∴grey-white or clay-coloured stools. The conjugated bilirubin cannot be excreted into bile; therefore it backs up into the blood and spills into the urine. Therefore will measure a high serum level of conjugated (direct) bilirubin and conjugated (direct) bilirubin will be present in urine.

3. Alkaline phosphatase (Alk phos)

- Enzyme produced mainly in liver and bone (but also in kidney, intestine, placenta)
- Excreted by liver into bile, therefore sensitive indicator of biliary obstruction
- Also good indicator of liver space lesions, e.g., carcinoma
- Not specific - level may increase with increased bone osteoblast activity, e.g., hyperparathyroidism
- Five isoenzymes

4. SGOT (AST)

- Serum glutamic - oxaloacetic transaminase aspartate transaminase
- Enzyme found mostly in heart and liver (but also skeletal muscle, pancreas, kidney)
- Increase in level proportional to extent of damage to heart or liver cells

5. LDH

- Lactic dehydrogenase
- A group of enzymes found mostly in heart and liver (but actually in all metabolising cells)
- Not very sensitive and not specific
- Can differentiate where cell damage is occurring by examining the isoenzyme pattern

6. SGPT (ALT)
- Serum glutamic pyruric transaminase
- Enzyme found liver, muscle, brain, other tissues

7. Prothrombin Time (PT)
- Prothrombin synthesized in liver
- Only abnormal in very severe liver disease

8. Serum Proteins
- Total protein = albumin + globulins
- Serum albumin - chiefly synthesized in liver
- Serum albumin decreases in most acute and chronic liver disease

HEART

1. SGOT (AST)
- See liver function tests
- Levels rise 8-12 hours after an MI.

2. LDH
- See liver function tests.
- Levels rise 24-48 hours after an MI.
- More sensitive than SGOT

3. CPK
- Creatine phosphokinase = CK = creatine kinase
- Enzyme found in heart muscle, skeletal muscle, brain
- First enzyme level to rise after an acute MI (in 2-6 hours)
- No change with liver damage, but level can increase with strenuous exercise, muscle injury, or often with **intramuscular injection**
- Isoenzymes

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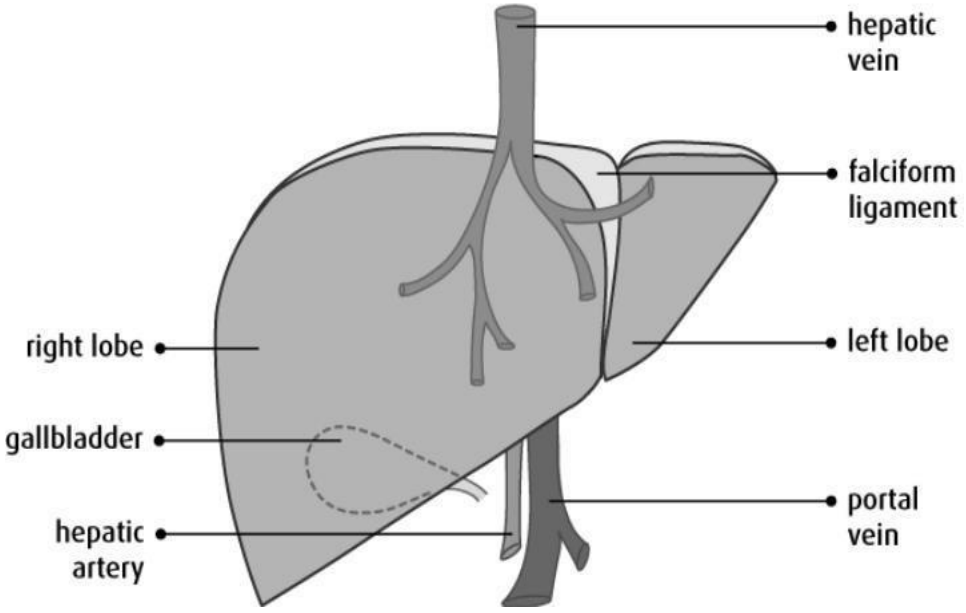
LIVER FUNCTION TESTS



LIVER FUNCTION TESTS

Liver: Liver is the largest gland of the body weighing 1.2-1.5 kg in an adult human. It is situated in the abdominal cavity, just below the diaphragm and has 2 lobes. The hepatic lobules are the structural and functional units of liver containing hepatocytes, arranged in the form of cords. Liver secretes bile which is alkaline, yellowish green fluid. It has no enzymes but help in emulsification of fats due to the presence of bile salts.

Major functions of liver:

1. **Metabolic functions:** Liver actively participates in carbohydrate, lipid, protein, mineral and vitamin metabolisms.
2. **Excretory functions:** Bile pigments, bile salts and cholesterol are excreted in the bile into intestine.

3. **Protective functions and detoxification:** Kupffer cells of liver perform phagocytosis to eliminate foreign compounds. Ammonia is detoxified to urea. Liver is responsible for metabolism of xenobiotic (detoxification).

4. **Haematological functions:** Liver participates in the formation of blood (particularly in the embryo), synthesis of plasma proteins (including blood clotting factors) & destruction of erythrocytes.

5. **Storage functions:** Glycogen, vitamins A, D & B_{12} and trace elements are stored in liver.

Tests to assess liver function:

The liver function tests (LFTs) are the biochemical investigation to assess the capacity of the liver to carry out any of the functions it performs. LFT will help to detect the abnormalities and the extent of liver damage. Two important facts should borne in mind while carrying out LFT:

i) Liver is a large size factory of safety. Therefore, it can perform many of it's functions almost normally, despite of the damage. ii) Selection of the right test is important in LFT. This is due to the fact that since liver participates in several functions, the function that is measured in LFT may not be the one that is adversely affected.

Classification of liver function tests:

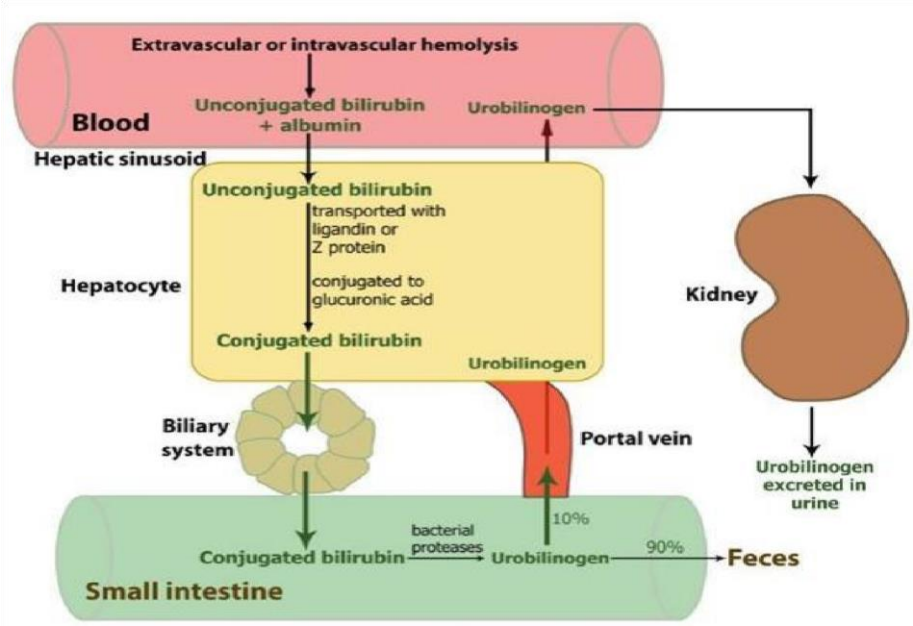
They are classified based on the major functions of liver.

i) **Tests based on excretory function:** Measurement of bile pigments, bile salts, bromosulphthalein test. ii) **Tests based on serum enzymes:** Determination of transaminases (ALT, AST), alkaline phosphatase (ALP), gamma glutamyl transpeptidase, 5'-nucleotidase and others. iii) **Tests based on synthetic function:** Serum proteins (albumin, globulins), prothrombin time. iv) **Tests based on metabolic capacity:** Galactose tolerance test, antipyrine clearance.

v) **Tests based on detoxification:** Hippuric acid synthesis.

1. Tests based on excretory function:

a) Bilirubin: Bilirubin is a bile pigment and is the excretory end product of heme degradation. It is conjugated in the liver to form bilirubin diglucuronide and excreted in bile.



Metabolism of bilirubin

Normal range: The normal concentration of serum bilirubin is in the range of 0.2-1.0mg/dl. Of this, the conjugated bilirubin (diglucuronide 75%; monoglucuronide 25%) is about 0.2-0.4mg/dl, while the unconjugated bilirubin is 0.2-0.6mg/dl.

Van den Bergh reaction: This is a specific reaction to identify the increase in serum bilirubin. Normal serum gives a negative Van den Bergh reaction.

Mechanism of the reaction: Van den Bergh reagent is a mixture of equal volumes of sulfanilic acid (in dilute Hcl) & sodium nitrite. The principle of the reaction is that diazotised sulfanilic acid reacts with bilirubin to form a purple coloured azobilirubin.

Direct and indirect reaction: Bilirubin as such is insoluble in water while the conjugated bilirubin is soluble. Van den Bergh reagent reacts with conjugated bilirubin and gives a purple colour immediately

(normally within 30 seconds). This is referred to as “direct positive Van den Bergh reaction”.

Addition of methanol (or alcohol) dissolves the unconjugated bilirubin which then gives the Van den Bergh reaction (normally within 30 minutes) positive and this is referred to as “indirect positive”. If the serum contains both conjugated and unconjugated bilirubin in high concentration, the purple colour is produced immediately (direct positive) which is further intensified by the addition of alcohol (indirect positive). This type of reaction is known as biphasic.

Uses: This reaction is highly useful in understanding the nature of jaundice. This is due to the fact that the type of jaundice is characterised by increased serum concentration of unconjugated bilirubin (haemolytic), conjugated bilirubin (obstructive) or both of them (hepatic). Therefore, the response of Van den Bergh reaction can differentiate the jaundice as follows.

- Indirect positive - Haemolytic jaundice
- Direct positive - Obstructive jaundice
- Biphasic - Hepatic jaundice

The conjugated bilirubin, being water soluble, is excreted in urine. This is in contrast to unconjugated bilirubin which is not excreted. Bilirubin in urine can be detected by “Fouchet's test” or “Gmelin's test”.

b) Bromosulphthalein test: Bromosulphthalein is a dye used to assess the excretory function of liver. It is a non-toxic compound and exclusively excreted by the liver (through bile). BSP is administered intravenously (5mg/kg body weight) and it's serum concentration is measured at 45 minutes and at 2 hours. In normal individuals, less than 5% of the dye is retained at the end of 45 minutes. Any impairment in liver function causes an increased retention of the dye. This test is quite sensitive to assess liver abnormality with particular reference to excretory function.

2. Tests based on serum enzymes:

Liver cells contain several enzymes which may be released into the circulation in liver damage. Measurement of selected enzymes in serum is often used to assess the liver function. It must be noted that there is no single enzyme that is absolutely specific to liver alone. Despite this fact, serum enzymes provide valuable information for LFT. Some of these enzymes are:

a) Transaminases (SGOT/AST & SGPT/ALT)
b) Alkaline phosphatase (ALP)
c) Gamma glutamyl transpeptidase (GGT)
d) 5'-nucleotidase
e) Others

a) Transaminases: The activities of two enzymes namely- serum glutamate pyruvate transaminase (SGPT; recently called as alanine transaminase-ALT) and serum glutamate oxaloacetate transaminase (SGOT; recently known as aspartate transaminase-AST) are widely used to assess the liver function. ALT is a cytoplasmic enzyme while AST is found in both cytoplasm and mitochondria. The activity of these enzymes is low in normal serum (ALT 5-40IU/L; AST 5-45IU/L). Serum ALT and AST are increased in liver damage. However, ALT is more sensitive and reliable for the assessment of LFT.

Estimation of serum transaminases cannot identify the causes of hepatic damage. Further, they don't have much prognostic value.

b) Alkaline phosphatase (ALP): It is mainly derived from bone and liver (the cells lining the bile canaliculi). A rise in serum ALP (normal 3-13 KA units/dl), usually associated with elevated serum bilirubin is an indicator of biliary obstruction (obstructive/post hepatic jaundice). ALP is also elevated in cirrhosis of liver and hepatic tumours.

Liver is not the sole source of alkaline phosphatase. Therefore, it's measurement has to be carefully viewed before arriving at any conclusion. The liver and bone isoenzymes of ALP can be separated by electrophoresis.

c) Gamma glutamyl transpeptidase (GGT): This is a microsomal enzyme widely distributed in body tissues, including liver. Measurement of GGT activity provides a sensitive index to assess liver abnormality. The activity of this enzyme almost parallels that of transaminases in hepatic damage. Serum GGT is highly elevated (normal 5-40IU/L) in biliary obstruction and alcoholism. Further, several drugs (e.g. phenytoin) induce and increase this enzyme in circulation).

d) 5'-nucleotidase: The serum activity of 5'-nucleotidase (normal 215U/L) is elevated in hepatobiliary decrease and this parallels ALP. The advantage with 5'-nucleotidase is that it is not altered in bone disease.

e) Others: Serum isocitrate dehydrogenase and isoenzymes of lactate dehydrogenase (LDH_4 and LDH_5) are also useful in LFT.

Interpretation of result:

Very often, a combination of serum enzyme estimations (instead of a single one) is used for a better understanding of liver functions. For instance, a large increase in transaminases (particularly ALT) relative to a small increase in alkaline phosphatase indicates hepatocellular damage. On the other hand, a small increase in transaminases and a large increase in alkaline phosphatase shows biliary obstruction.

3. Tests based on synthetic function:

a) Serum proteins: Albumin is solely synthesized by the liver. It has a half-life of about 20-25 days, therefore it is a good marker to assess chronic (and not acute) liver damage. Low serum albumin is commonly observed in patients with severe liver damage. It must be noted that the serum albumin concentration is also decreased due to other factors such as malnutrition.

Functional impairment of liver is frequently associated with increased synthesis of globulins. Cirrhosis of the liver causes a reversal of albumin/globulin ratio (A/G ratio). Serum electrophoresis of proteins reveals increased albumin and decreased gamma globulin concentration. This may not have much diagnostic importance since several diseases are associated with altered electrophoretic pattern of serum proteins.

b) Prothrombin time: The liver synthesizes all the factors concerned with blood clotting. A decrease in concentration of plasma clotting factors is found in the impairment of liver function. This can be assessed in the laboratory by measuring prothrombin time which is prolonged in patients with liver damage, compared to normal. The halflives of clotting factors are relatively short (5-72 hrs.), therefore, changes in prothrombin time occur quickly. Hence, this test is useful to assess acute as well as chronic liver damages; besides it's help in the prognosis.

Vitamin K is required for the synthesis of blood clotting factors II, VII, IX and X. Therefore, vitamin K deficiency can also cause prolonged prothrombin time which must be ruled out, before drawing conclusions on the liver functions. This is done by measuring prothrombin time before and after administration of vitamin K.

4. Tests based on metabolic capacity:

Galactose tolerance test: Galactose is a monosaccharide, almost exclusively metabolized by the liver. The liver function can be assessed by measuring the utilisation of galactose. This is referred to as galactose tolerance test.

Procedure: The subject is given intravenous administration of galactose (about 300mg/kg body weight). Blood is drawn at 10 minute intervals for the next 2 hours and galactose estimated. In the normal individuals, the half-life of galactose is about 10-15 minutes. This is markedly elevated in hepatocellular damage (infective hepatitis, cirrhosis).

5. Tests based on detoxification:

Hippuric acid synthesis: The liver is the major site for the metabolism of xenobiotics (detoxification). Measurement of hippuric acid synthesis is an ideal test for assessing the detoxification function of liver. Hippuric acid is produced in the liver when benzoic acid combines with glycine.

Procedure: About 6g of sodium benzoate (dissolved in about 250ml water), is orally given to the subject, after a light breakfast (usually 2hrs later) and after emptying the bladder. Urine collections are made for the next 4 hours and the amount of hippuric acid excreted is estimated. Theoretically, 6g of sodium benzoate should yield 7.5g of hippuric acid. In the healthy persons, about 60% of sodium benzoate (equivalent to 4.5g hippuric acid) is excreted in urine. A reduction in hippuric acid excretion (particularly <3g) indicates hepatic damage.

Conclusion:

The choice of biochemical tests to measure liver functions mostly depends on the purpose of the investigation. The clinical history of the subject is often a guiding factor in this regard. A single test in isolation may have a little diagnostic value.

Frequently, a combination of laboratory investigations are employed in LFT. These include serum bilirubin (conjugated and unconjugated), ALT, AST, ALP, GGT and proteins (albumin, globulins).

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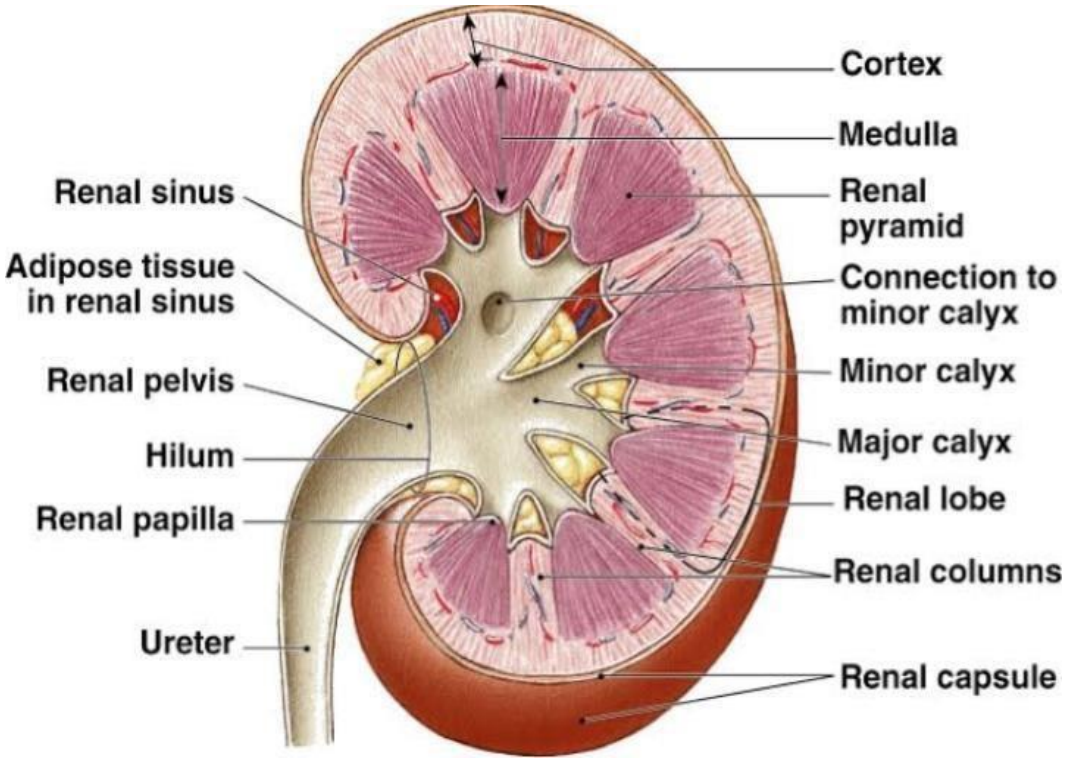
RENAL FUNCTION TESTS



RENAL FUNCTION TESTS

Kidney: The kidneys are the vital organs of the body, performing the following major functions.

1. **Maintenance of homeostasis:** The kidneys are largely responsible for the regulation of water, electrolyte and acid-base balance in the body.
2. **Excretion of metabolic waste products:** The end products of protein and nucleic acid metabolism are eliminated from the body. These include urea, creatinine, uric acid, sulphate and phosphate.
3. **Retention of substances vital to body:** The kidneys reabsorb and retain several substances of biochemical importance in the body.

e.g. glucose, amino acids etc.

4. **Hormonal functions:** The kidneys also function as endocrine organs by producing hormones.
    - Erythropoietin, a peptide hormone, stimulates haemoglobin synthesis and formation of erythrocytes.
    - 1,25-Dihydroxycholecalciferol (calcitriol), the biochemically active form of vitamin-D is finally produced in the kidney. It regulates calcium absorption from the gut.
    - Renin, a proteolytic enzyme liberated by kidney, stimulates the formation of angiotensin II which, in turn, leads to aldosterone production. Angiotensin II and aldosterone are the hormones involved in the regulation of electrolyte balance.

Nephron: The main functional unit of kidney is nephron, each kidney is composed of approximately 1 million nephrons. The blood supply to kidneys is relatively large. About 1200ml of blood (650ml plasma) passes through the kidneys, every minute. From this, about 120-125ml is filtered per minute by the kidneys and this is referred to as "glomerular filtration rate (GFR)". The process of urine formation basically involves 3 steps.

1. Glomerular filtration.
2. Tubular reabsorption.
3. Tubular secretion.

Purpose of renal function tests:

1. To diagnose the disease or disorders of kidneys and urinary tract and other systematic diseases that effect the kidney functioning.
2. Urine examination is usually employed to diagnose most of the kidney disorders.

Classification of renal function tests: Renal function tests may be divided into 4 groups.

1. **Urine examination:** This is classified into 3 types.
    - **a) Physical/macroscopic examination:** It include volume, colour, odour, reaction (P^H), specific gravity.
    - **b) Biochemical examination:** It include proteins, sugars, ketone bodies, bile salts, bile pigments, blood.
    - **c) Microscopic examination:** It include cells, crystals, casts, microorganism, parasites, contamination.

2. **Glomerular function tests (clearance tests):** All the clearance tests (inulin, creatinine, urea) are included in this group.

3. **Analysis of blood/serum:** Estimation of blood urea, serum creatinine, protein and electrolyte are often useful to assess renal function.

4. **Tubular function tests:** Urine concentration test, urine dilution test, urine acidification test.

1. Urine examination:

a) Physical/macroscopic examination:

i) volume:

Normal volume is 1000-2000/24hrs (depending upon water intake). Increase in urine levels id known as polyuria (more than 2000ml/day). This may be due to certain drugs, IV solutions, diabetes mellitus etc. Decrease in urine levels is known as oliguria (less than 400ml/day).
Less than 100ml of urine in 24hrs is known as anuria. These conditions may be due to dehydration, congestive heart failure, due to renal tract obstruction. **ii) Colour:**

The normal colour of urine is pale yellow to urochrome. Abnormal colours are observed during diseased state. Various colours and corresponding diseases are as follows.

- Red: haematuria
- Blue: cholera
- Yellow-brown: bilirubin
- Yellow-green: biliverdin
- Orange: excessive sweat, fever
- Black: melanin **iii) Odour:**

Smell of urine is usually light pungent. Various other smells are also observed.

- Aromatic: volatile fatty acids
- Ammonical: due to bacterial action
- Fruity odour: ketonuria **iv) P^H:**

Normal P^H of urine is 6, it may be between 5-9 depending upon diet. High P^H (classic renal alkalosis) is due to sodium carbonate containing drugs, strict vegetarian. Lower P^H (classic renal acidosis) is due to ammonium chloride containing drugs, starvation, ketosis, fever, diabetes.

v) Specific gravity:

Normal value of specific gravity 1.001-1.040. Increase in specific gravity is due to dehydration, fever, vomiting, diarrhoea, congestive heart failure, diabetes mellitus. Decrease in specific gravity is due to diabetes insipidus.

b) Biochemical examination:

i) Proteins:

Test	Observation	Inference
Sulphosalicylic acid test: 3ml of urine + sulphosalicylic acid drop by drop	White precipitation is formed	Presence of proteins
Heller's nitric acid test: 3ml concentration of nitric acid + urine sample drop wise from sides of test tube	White ring is formed at the junction of two layers	Presence of proteins
Heat coagulation: Boil 5ml of urine sample for 5 minutes	Turbidity is observed	Presence of proteins

Presence of proteins in urine is called proteinuria. Various causes of proteinuria are severe exercise, high protein diet, pregnancy, kidney disease, damage to lower urinary tract, fasting. **ii) Sugars:**

Test	Observation	Inference

Benedict's reagent test: 2ml of urine + 2ml of benedict's qualitative reagent, boil for 2ml and cool.	a) Green precipitate b) Brick red precipitate c) Yellow precipitate	a) 1% glucose b) 2% glucose c) more than 2% glucose
Fehling's reagent test: 2ml of Fehling's A and Fehling's B + 2ml of urine, boil for 2-5 minutes	Red or yellow precipitate is formed	Presence of glucose

Presence of glucose in urine is called glucosuria, this may be due to diabetes mellitus, hypertension, corticosterol drugs etc.

iii) Ketones:

Test	Observation	Inference
Rothera's test: 5ml of urine + small amount of ammonium sulphate + 2 drops of sodium nitroprusside + 2ml of strong ammonia solution and wait for 10 minutes	Permanganate colour is observed	Presence of ketone bodies

Presence of ketone bodies in urine is called ketonuria, this may be due to starvation, excessive fatty acid metabolism, pregnancy. **iv) Bile salts:**

Test	Observation	Inference
Sulphur powder test: Take 5ml of urine in a beaker and sprinkle sublimed sulphur powder	Powder sinks to bottom	Presence of bile salts

Presence of bile salts in urine may be due to jaundice, obstruction of biliary tract, liver diseases. **v) Bile pigments:**

Test	Observation	Inference
Gmelin's test: 10ml of urine + 2 to 3 drops of dilute Hcl, filter it, allow the filter paper to dry and put a drop of HNO_3	Colouration of paper in following order: Green, blue, violet, red, yellowish red	Presence of bile pigments
Heller's nitric acid test: 3ml of conc. HNO_3 + add urine drop wise slowly	Fine ply of colours is observed	Presence of bile pigments

Apart from these tests Van den Bergh's reaction, Fouchet's test are also employed. Presence of bile pigments in urine may be due to jaundice (haemolytic or obstructive), obstruction of biliary tract, liver diseases. **vi) Blood:**

Test	Observation	Inference
Benzidine test: 2ml of urine + few ml of H_2O_2 + pinch of benzidine in acetic acid	Green colour is observed	Presence of blood

Presence of blood in urine is called haematuria, various causes of haematuria are renal calculi, glomerulonephritis, severe urinary tract infections, renal tract tumour.

c) Microscopic examination:

In this, the analysis is done simply by pouring the urine sample into test tube and centrifuging it for 5 minutes, the top liquid part is discarded and the sediment left at the bottom is mixed with 1 drop of urine and is studied under a microscope. **i) Cells:**

Few epithelial cells & RBC are observed occasionally in urine. This may be due to fever or urinary tract calculi or acute renal damage or glomerulonephritis. **ii) Crystals:**

Various crystals are found in the acidic urine like uric acid crystals, calcium oxalate crystals, cysteine crystals etc. Crystals found in basic urine are carbonate and phosphate crystals. **iii) Casts:**

Urinary casts are cylindrical structures produced by the kidney and present in the urine in certain disease states. They form in the proximal convoluted tubule & collecting duct of nephrons, then dislodge and pass into the urine, where they can be detected by microscopy. These casts are of 2 types.

Cellular casts	**Acellular casts**
RBC casts WBC casts Epithelial casts	Hyaline casts Fatty casts Waxy casts Pigment casts

2.Glomerular filtration tests (clearance tests):

a) Creatinine clearance: Creatinine is an excretory product derived from creatine phosphate (largely present in muscle). The excretion of creatinine is rather constant and is not influenced by the body metabolism or dietary factors. Creatinine is filtered by the glomeruli and only marginally secreted by the tubules. The value of creatinine clearance is close to GFR, hence it's measurement is a sensitive and good approach to assess the renal glomerular function.

Definition: Creatinine clearance may be defined as the volume (ml) of plasma that would be completely cleared of creatinine per minute.

Procedure: In the traditional method, creatinine content of a 24 hour urine collection and the plasma concentration in this period are estimated. The creatinine clearance (C) can be calculated as follows:

$$C = \frac{U \times V}{P}$$

Where;

U = Urine concentration of creatinine (mg/dl or mmol/L)

V = Volume output in ml/min (24hr urine volume divided by 24×60)

P = Plasma concentration of creatinine (mg/dl or mmol/L)

Reference values: The normal range of creatinine clearance is around 120-145ml/min. These values are slightly lower in women. In recent years, creatinine clearance is expressed in terms of body surface area.

Diagnostic importance: A decrease in creatinine clearance value (<75% normal) serves as sensitive indicator of decreased GFR, due to renal damage. This test is useful for an early detection of impairment in kidney function.

b) Urea clearance: Urea is the end product of protein metabolism. After being filtered by the glomeruli, it is particularly reabsorbed by the renal tubules. Hence, urea clearance is less than the GFR and further it is influenced by the protein content of diet. For these reasons, urea clearance is not as sensitive as creatinine clearance for assessing renal function. Despite this fact, several laboratories traditionally use this test.

Definition: Urea clearance is defined as the volume (ml) of plasma that would completely cleared of urea per minute. It is calculated by the formula:

$$C_m = \frac{U \times V}{P}$$

Where;

C_m= Maximum urea concentration

U = Urea concentration in urine (mg/ml) V = Urine excreted per minute in ml.

P = Urea concentration in plasma (mg/ml)

The above calculation is applicable if the output of urine is more than 2ml/min. This is referred to as maximum urea clearance and the normal value is around 75ml/min.

Interpretation of result:

- If urea concentration is >70%, renal function is normal.
- If urea concentration is between 40-70%, renal function is mildly impaired.
- If urea concentration is <20%, renal function is severely impaired.

3. Analysis of blood/serum: Estimation of serum creatinine and blood urea are useful. These tests are less sensitive than the clearance tests. Serum creatinine is a better indicator of urea.

a) Blood urea:

Urea is major nitrogenous end product of protein and amino acid catabolism, produced by liver and distributed throughout intracellular and extracellular fluid. Urea is filtered freely by the glomeruli. The reference value for serum urea is 10-40mg/dl.

Many renal diseases with various glomerular, tubular, interstitial or vascular damage can cause an increase in urea concentration. High protein diet causes significant increase in plasma urea concentration and urinary excretion.

b) Serum creatinine:

Creatinine is formed from creatine in muscles. It is released in blood and is excreted by kidneys in urine. Normal range of serum creatinine is 0.6-1.5mg/dl. Glomerular filtration is reduced in renal failure. This causes retention of creatinine in blood. Hence, serum creatinine is raised in renal failure. Serum creatinine is a better indicator of glomerular function than serum urea.

4. Tubular function tests:

a) Urine concentration test:

This is used to assess the renal tubular function. This test includes the measurement of specific gravity which depends on concentration of solute present in urine. Normal specific gravity value is 1.020. **b) Urine dilution test:**

Urine dilution test is done to assess the ability of kidneys to eliminate water. This function is tested by measuring urinary output after ingesting a large volume of water. This test is not advisable for patients with adrenal insufficiency. **c) Urine acidification test:**

Urine acidification test is done to check the ability of kidney to produce acidic urine which is the function of tubules. For this test, patient is asked to empty the bladder. The P^H of urine is measured which

should be between 4.6-5.0. In patients with renal tubular acidosis, P^H does not fall below 5.3 even after dose of ammonium chloride.

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