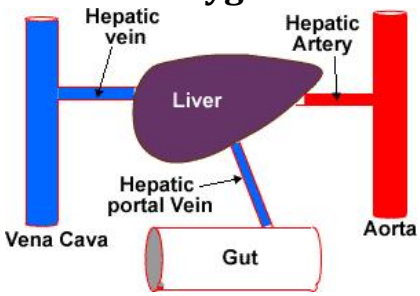
Clinical Biochemistry

Liver and liver function test

The liver is the largest gland in the body; it is multifunctional. To understand the function of the liver it is necessary to understand the blood supply to the liver. **It is supplied by the hepatic artery in the typical manner but it is the only digestive organ drained by the inferior vena cava**. Other digestive organs such as the small intestine, parts of the large intestine, stomach and pancreas are drained by the hepatic portal system which takes the blood directly to the liver. **Thus, the liver receives oxygen poor, nutrient rich blood from the hepatic portal system and oxygen rich blood from the hepatic artery.**



Functions of the liver

a. Digestive and Metabolic Functions:

1. Synthesis and secretion of bilirubin, bile acids and bile.
2. Storage of glycogen and lipid reserves.
3. Maintaining normal blood glucose, amino acid and fatty acid concentrations.
4. Synthesis and release of cholesterol.
5. Inactivation of toxins.
6. Storage of iron reserves.
7. Storage of fat-soluble vitamins.
8. Protein metabolism, formation of urea from ammonia produced from amino acid metabolism.

b. **Non-Digestive Functions:**

a. Synthesis of plasma proteins.
b. Synthesis of clotting factors.
c. Synthesis of the inactive angiotensinogen .
d. phagocytosis of damaged red blood cells.
e. Storage of blood.
f. Breakdown of circulating hormones (insulin and epinephrine) and immunoglobulins.
g. Inactivation of lipid-soluble drugs.

I. Changes in level of certain Serum enzymes

The liver secretes specific enzymes for performing its multitasking activity, when the liver is malfunctioning, these enzymes often get elevated, and in hepatic failure they are normal or subnormal.

A. Enzymes indicating hepato-cellular injury and repair (Leakage enzymes).

1. Alanine aminotransferase (ALT)or serum glutamic pyruvic transaminase(SGPT):

It is a liver specific enzyme in man, primates, dog and cat; it is not used in large animals because it is found in very low concentration in their hepatocytes. It is found in the cytoplasm of the hepatocytes therefore it is released following severe, acute and diffuse hepato - cellular necrosis.

Interpretation: Elevation in serum level is considered significant when it increases two to three times above normal; it remains elevated for 1-3 weeks in transient acute cases,

Mild to moderate increase in ALT may indicate none hepatic disorders like cardiac failure, inflammatory GI diseases.

2. Aspartate aminotransferase (AST) or serum glutamic oxaloacetic transaminase (SGOT):

It is found in the mitochondria of liver cells in all animals, It is not liver specific enzyme because it is found in all tissues of the body, used to detect destruction in a wide variety of tissues e. g.: skeletal and cardiac muscles together with Creatine Kinase (CK or CPK).

Interpretation:

- When there is increase in AST with normal CK, this indicates liver injury.
- When increase in ALT is much more than AST + normal CK = liver problems.
- When increase in AST is much more than ALT+ Increase in CK = muscle injury or both hepatic and muscular injury.

3. Sorbitol dehydrogenase (SDH):

It is used to diagnose liver dysfunction in all animals especially those with low ALT like equine. Although it is found in many organs the highest concentration is found in the liver,

Interpretation: Immediate and marked increase in the serum in response to acute liver injury. It usually return to normal in less than 24 hours, so it is not used to detect chronic hepatic diseases, its level should be measured immediately after collection.

4. Arginase:

It is found in liver of all animals, within the mitochondria

Interpretation: When it is increased it indicates severe hepatic diseases in all animal species, especially those lacking ALT.

When regeneration of liver tissue started it will return to normal level quickly (short half life in the serum),

If it remains high it indicates progressive necrosis and predict poor prognosis in all mammals.

5. Glutamic dehydrogenase (GD):

It is found in high concentration in the mitochondria of liver cells.

Interpretation: If it increases it indicates severe liver damage in all animal species especially ruminants.

6. Ornithine carbamyl transferase (OCT):

Liver specific enzyme in all animal species.

Interpretation: Its elevation indicates chronic active hepatitis and hepatic necrosis and not when healing is taking place.

B. Liver enzymes that act as markers for cholestasis:

They are two enzymes; both show minimal activity in normal hepatic tissue, markedly increased in plasma subsequent to increase in their production stimulated mostly by impaired bile flow that irritates the lining mucosa.

1) Alkaline phosphatase (ALP, SAP):

- It is produced by cells lining the canalicular membrane of hepatocytes, also it is present in all tissues of the body (liver, bone, kidney, intestine, placenta). ALP is popular in small animals, less in large animals due to very wide range and fluctuation in its normal hepatic values. Have short half life in the blood (< 6 min) except hepatic enzymes.

Interpretation:

- Slight increase in ALP indicates hepatic necrosis due to intra-hepatic billiary obstruction.
- Hepatic necrosis leads to marked increase in ALT+ moderate increase in ALP + no change in GGT.
- Bile duct obstruction leads to marked increase in ALP and GGT+ moderate increase in leakage enzymes.
- Bone neoplasia, bone growth, and in late pregnancy.

2) Gamma glutamyl transferase (GGT):

It is a valuable serum marker for disorders of the hepato-biliary system resulting in cholestasis. It is widely used in all animals, especially farm animals instead of ALP, present in other tissues but its activity is highest in the canalicular surface of hepatocytes and epithelial lining of bile ductules and ducts.

Interpretation: Cholestasis

Which is better ALP or GGT? It depends on animal species, in canine ALP is more sensitive than GGT. **Why?** Because ALP is present in higher concentration in their livers (three times more than its con. in cats liver). Cats, although have lower ALP in their livers in addition to short half life in the circulation, GGT and ALP are of equal importance in assessing cholestasis in them. Cattle, sheep and horses, have greater liver and serum GGT activity than pet animals, in addition to that their ALP has a very wide normal range + fluctuation in its serum level, so GGT is more useful in such animals.

How to differentiate between acute and chronic hepatic injury?

1) Acute hepatocellular injury:

- **In small animals:** there is marked increase (>50-100 times the normal) in ALT and AST enzymes + slight to moderate increase in ALP + little or no increase in GGT.
- **In ruminants and horses:** marked increase in SDH and AST + variable (mild- moderate) increase in GGT.

In extra- hepatic bile duct obstruction or in chronic fibrotic liver diseases, there is increase in ALP and GGT with normal or only slightly increased leakage enzymes.

II. Biochemical tests depending on evaluating hepatocytes activity in uptake,processing and excretion of :

1. Endogenous substances that includes billirubin (bile pigment) and bile acids.
2. Exogenous dye excretion tests.

1. Endogenous substances: Found normally in the serum but it increases in liver diseases

a. Billirubin (BR): Its level in man and most animals is < 0.5 mg/dl; in horses it is about 2 mg/dl that is why plasma of horses is more yellowish. Plasma BR concentration is directly proportional to its production from RBC destruction and inversely related to hepatic clearance.

Plasma BR clearance depends on its:

a. Hepatic uptake.
b. Hepatic conjugation of bilirubin to glucuronides in hepatic microsomes.
C. Its excretion with the bile.

. Plasma BR is a relatively insensitive test because of the large reserve capacity of the liver, but when hyperbilirubinaemia is present, its degree and fluctuation are valuable both in diagnosis and prognosis.

Jaundice: is a clinical sign characterized by yellowish colouration of the skin and mucous membranes due to hyperbilirubinaemia.

Kinds of jaundice or Icterus:

1- Prehepatic (Hemolytic), due to excessive haemolysis or a disorder that leads to decrease in the clearance of plasma billirubin e. g. fasting in normal horses or certain inherited disease.UBR is predominantly increased in the serum (except in most canine and feline CBR increased more than UBR).

2- Hepatic (intrahepatic lesion and/ or obstruction), it is associated with increase in CBR (except in horses) it is due to functional defect or mechanical obstruction of biliary canaliculi in the liver, CBR regurgitate into the serum. Lesser and varying amounts of UBR may increase in the serum due to defective hepatic uptake of pigment.

3- Extra hepatic (post hepatic), it is due to obstruction of the main bile duct due to either parasitic or bacterial infections in the area, stones neoplastic lesions are important causes of obstructive jaundice. It is

characterized by increase in CBR in the serum of the patient (except in equine UBR is predominantly increased in obstructive jaundice). In ruminants, increase in BR is not a sensitive indicator for hepatic dysfunction, it increases in late stages of diffuse hepatic diseases, high level of UBR is found in the serum of ruminants in haemolytic anaemia.

b.Bile acid (BA):

They are synthesized in the liver from cholesterol, their Na and K salts are present in the bile, play important role in the digestion of fat in the small intestine, there are two types of BA, chenodeoxycholic acid (chenic acid) and cholic acid. High proportion of BA is reabsorbed and recycled to the liver and resecreted in the bile, only small quantity escape to peripheral blood.
Fasting BA (FBA) is less than postprandial BA(PPBA) because BA are stored in the gall bladder, feeding induces contraction of gall bladder, release of bile, to the intestine then reabsorbed to the portal circulation and taken by the hepatocytes, few amount enter the circulation.
Measurement of BA is easy to perform, highly sensitive and can detect about 40-50% decrease in liver normal function of small animals. In ruminants it is not used because of wide normal range reported in these animals.

Interpretation of increase in serum BA:-

- Reduced hepatic functional mass as in hepatic lipidosis.
- Post and intrahepatic biliary obstruction that leads to regurgitation of BA to blood.
- Portosystemic (PSS), which means shunting of portal blood to the systemic circulation before it passes to the liver through fetal vascular orifices that should be closed after birth. It is either congenital or acquired due to long standing portal hypertension.

2- Exogenous dye excretion tests:

To check the ability of the liver to excrete foreign dyes introduced to the body and they are mainly eliminated by the liver.

a. Bromosulfophthalein (BSP): It is an organic dye, inoculated i. v., taken by hepatocytes, conjugated and excreted with the bile in a path way similar to that of billirubin.

Protocol: i) Fast the animal. ii) Inject 5mg/kg body weight. iii) After 30 min. determine the level of BSP in the plasma and calculate % of retention.

Normal: In dogs<5%, in sheep and goat 6% retention is normal (collect blood after 10 min).

b. Indocyanine green clearance (ICG): It is used in human as a substitute to BSP.
Protocol: i) Fast the animal. ii) Inject i. v., 1.5-0.8 mg/kg. iii) Collect plasma at, o time and after 5, 10, 15, 30 min.post injection. iv) Measure the level of the dye in all samples and determine the half life of clearance (T1/2).which is the time needed to excrete half of the dye injected.
Normal: T1/2 in dogs 8 min., in horses 3.5 min.
30 min retention in Normal fasted dog: <7-14%, in fasting cat :< 3-7%.

- **Interpretation of increase in the T1/2 of BSP and ICG clearance and /or decrease in the percentage of their excretion may indicate either:**

1. Hyperbillirubinaemia (competition for the same receptors on the hepatocytes).
2. Decrease in hepatic blood flow.
3. Hepatic necrosis.
4. Diffuse liver fibrosis.
5. Post-hepatic obstruction.

The ICG test is superior to BSP test in detecting liver diseases in dogs.

III. Tests of hepatic synthetic ability:

a. Ammonia tolerance test: The liver is responsible for the detoxification of ammonia produced from the catabolism of amino acids to urea that can be excreted through the kidneys.
severe liver disease may impair the this activity leading to increase in the level of ammonia in the body especially in brain tissue that causes hepatoencephalopathy producing nervous signs in the affected animal.
Random blood ammonia test may be normal, challenge the animal with exogenous ammonia either through mouth or better through a rectal enema, then follow blood ammonia level in the blood.

Hyperammonaemia may reveals (Interpretation):

1. Deficiency in urea cycle enzymes (with normal BA level) it is mostly a hereditary abnormality.
2. Abnormal portal blood flow (congenital) with normal liver enzymes.
3. Liver cirrhosis with increase in BA and liver enzymes.

b. Blood urea nitrogen (BUN): There is decrease in the level of blood urea with normal serum creatinine (SCr) and increase in blood ammonia.

c. Cholesterol: In general altered lipid metabolism is associated with liver diseases, but it is not sensitive. Cholesterol is eliminated by the formation of BA, so in severe hepatic malfunction hypercholestero- laemia develops.

d. Coagulation factors: - All clotting factors are synthesized in the liver; diffuse, severe hepatic injury will lead to decrease in their concentration in the blood. Prothrombin has a very short half life in the circulation; it is an early indicator of hepatic dysfunction with increase in PT and PTT, in addition to that vit. K dependent clotting factors may undergo acute decrease in their activity (factor II, VII, IX, X).

e. Plasma protein: Albumin is the most abundant plasma protein, most animals have tremendous reserve for its synthesis,>80-90% loss of liver activity is needed to have hypoalbuminaemia. Acute hepatitis is associated with normal serum albumin, because it has a long half life, while in chronic hepatitis there is a decrease in serum albumin, with increase in β and α globulins (due to continuous immunological stimulation caused by different kinds of antigens and foreign materials escaping hepatic removal).Increase in y globulin and / or decrease in albumin is typical for hepatic necrosis or cirrhosis.

f. Glucose: It is an insensitive liver function test; > 70% of liver activity must be lost till hypoglysaemia appeared. Hypoglysaemia is due to decrease in hepatic glycogen stores and decrease in insulin clearance.

g. Uric acid: The end product of purine metabolism in most mammals is allantoin, they have uricase enzyme in their livers (except man and Dalmatian dogs). Dogs affected with hepato- cellular jaundice (unlike haemolytic and post- hepatic jaundice) are associated with increase in blood uric acid, but it is not sensitive as ALT and FBA.

Clinical signs of hepatitis in small animals: All, some, or only one of these signs may be present.

1) Intermittent recurrent abdominal or gastrointestinal upsets. Loss of appetite, vomiting, diarrhea, constipation.
2) Progressive depression or lethargy. Does not want to play anymore or refuses to go for walks.
3) Swollen belly with a "fluid filled" look. This is also known as ascites and is actually fluid accumulation in the belly due to circulation alterations in the abdomen.

4) Pale gray feces. Bile pigments are what give poop it's characteristic brown colour and if the liver is not processing bile properly, the feces will not get their colour.
5) Orange urine. The improper processing of bile results in the excretion of bilirubin in the urine in high amounts, thus orange urine.
6) Jaundice, also known as icterus. Any pale or white skin or visible tissue takes on a yellow hue. Again the biliary pigments are accumulating in the body because the liver is not processing them.
7) Rarely: bleeding problems. Many of the proteins required for proper blood clotting are created in the liver. Remove these proteins and blood clotting decreases.
8) Hepatic encephalopathy or severe neurological signs. Behavioral changes, seizures, aimless pacing or circling, head pressing. May be associated with meal time.
9) Pain associated with the abdomen. This is due to the stretching of the liver capsule. May be noted when the dog is lifted around the belly or when the veterinarian probes (palpates) the abdomen. The veterinarian may also notice a swollen liver while palpating with some of the more acute liver diseases.
10) Chronic weight loss or wasting. The liver processes all the building blocks. If it fails to process, the body fails to maintain itself.
11) Increased water consumption and urination. Most likely due to dramatic shifts in serum and kidney salt balances. May be behavioral too.

Photosensitization: Phyloerythrin the normal breakdown product of chlorophyll, it is excreted normally with bile, when there is retarded excretion of bile it is accumulated in the skin causing photosensitization when the animal is exposed to direct sun light.