Assessment Of Liver Function
Analysis of Bilirubin (Method Selection)

- Unfortunately, no single method for the determination of bilirubin will meet all the requirements of the clinical laboratory
  - For the evaluation of jaundice in newborns (no lipochromes), the direct spectrophotometric method is satisfactory
  - The sources of error in this technique are turbidity, hemolysis, and yellow lipochrome pigments
  - Hemolysis and turbidity can be blanked out by measuring a second wavelength, but the yellow lipochromes cannot be blanked out.
  - In patients older than 1 month, a diazo-colorimetric procedure is necessary

jendrassik-Grof Method for Total and Conjugated Billirubin Determination

(indirect method)

- Serum or plasma is added to a solution of sodium acetate and caffeine-sodium benzoate, which is then added to diazotized sulfanilic acid to form **purple azobilirubin**
- The **sodium acetate** buffers the pH of the diazotization reaction, whereas the **caffeine-sodium benzoate** accelerates the coupling of bilirubin with diazotized sulfanilic acid
- This reaction is terminated by the addition of **ascorbic acid**, which destroys the excess diazo reagent
- A **strongly alkaline tartrate** solution is then added to convert the purple azobilirubin to blue azo-bilirubin, and the intensity of the color is read at 600 nm
- A **fasting** serum specimen, which is **neither hemolyzed** nor **lipemic**, is preferred.
- The specimen should be **kept in dark** after collection, analyzed within 3hrs, kept for 1 week in the refrigarator or 3 months in -20

Reference range

- Normal blood contains no conjugated bilirubin.
- Some conjugated bilirubin is reported as normal because current available methodology picks up some of the total bilirubin as a false positive
- For **adults**
  - Conjugated: 0-0.2 mg/dL (0-3 µmoI/L)
  - Unconjugated: 0.2-0.8 mg/dL (3-14 µmol/L)
  - Total: 0.2-1.0 mg/dL (3-17 µmol/L)
- For **infants**

INFANTS	PREMATURE, TOTAL	FULL TERM, TOTAL
24 hours	1–6 mg/dL	2–6 mg/dL
48 hours	6–8 mg/dL	6–7 mg/dL
3–5 days	10–12 mg/dL	4–6 mg/dL

Total - conjugated = unconjugated

(direct method) بطريقة اسمها بقيس conjugated bilirubin

(indirect method) بطريقة بقيس (conjugated or unconjugated) Total bilirubin

Direct Spectrophotometric Method for Determination of Total Bilirubin in Serum

- The absorbance of bilirubin in serum at 455 nm is proportional to its concentration ↑abs ↑Conc.
- The serum of newborns does not contain lipochromes, such as carotene, that would increase the absorbance at **455 nm**. The absorbance of hemoglobin at 455 nm is corrected by subtracting the absorbance at **575 nm**.
- Error will be introduced if the buffer is turbid. Because the method depends on the extinction coefficient of bilirubin, **all volumes must be accurate** and **cuvettes must be flat-surfaced**, with a path length of exactly 1 cm
- This method is relatively insensitive to hemolysis, which is often present in specimens obtained from infants, due to difficulty in skin puncture technique
- it is significantly affected by the presence of lipochromes and so cannot be used in infants older than a few months of age

Urobilinogen in Urine and Feces

- Urobilinogin is a colorless end product of bilirubin metabolism that is oxidized by intestinal bacteria to the brown pigment urobilin
- In the normal individual, part of the urobilinogen is excreted in the **feces**, and the remainder is reabsorbed into the portal blood and returned to the **liver.** A small portion that is not taken up by the hepatocytes is excreted by the **kidney** as urobilinogen
- Increased levels of urinary urobilinogen are found in hemolytic disease and in defective liver-cell function, such as hepatitis
- **Absence of urobilinogen** from the urine and stool is most often seen with complete **biliary obstruction.** Fecal urobilinogen is also decreased in biliary obstruction and in hepatocellular disease

gall bladder obstruction ⇐ urine بال urobilinogen اذا قلّ =

hemolysis ⇐ urine بال urobilinogen اذا علي =

Urobilinogen in Urine and Feces

- Most quantitative methods for urobilinogen are based on the reaction of this substance with **p-dimethylaminobenzaldehyde** to form a red color.
- Major improvements were made by using **alkaline ferrous hydroxide** to reduce urobilin to urobilinogen and added **sodium acetate** to eliminate interference from such compounds as indole
- The use of **petroleum ether** rather than diethyl ether for the extraction of urobilinogen was introduced to help in the removal of other interfering substances

Determination of Urine Urobilinogen (Semiquantitative)

- Principle.
  - Urobilinogen reacts with p-dimethyl aminobenzaldehyde (Ehrlich's reagent) to form a red color, which is then measured spectrophotometrically.
  - Ascorbic acid is added as a reducing agent to maintain urobilinogen in the reduced state.
  - The use of saturated sodium acetate stops the reaction and minimizes the combination of other chromogens with the Ehrlich's reagent.

- Specimen
  - A fresh 2-hour urine is collected which should be kept cool and protected from light.

Sources of Error

- The results of this test are reported in **Ehrlich units** rather than in milligrams of urobilinogen because of interfering substances

- Compounds, other than urobilinogen, that may be present in the urine and react with Ehrlich's reagent include porphobilinogen, sulfonamides, procaine, and 5-hydroxyindoleacetic acid. Bilirubin will form a green color and, therefore, must be removed, as previously described

- Fresh urine is necessary and the test must be performed without delay to prevent oxidation of urobilinogen to urobilin. Similarly the spectrophotometric readings should be made within 5 minutes after color production because the urobilinogen-aldehyde color slowly decreases in intensity.

Reference Range: Urine urobilinogen, 0.1-1.0 Ehrlich units/2 hr or 0.5-4.0 Ehrlich units/day (0.8 - 6.8 mmol/day); 1 Ehrlich unit is equivalent to approximately 1 mg of urobilinogen

Fecal Urobilinogen

- Visual inspection of the feces usually suffices to detect decreased urobilinogen.

- The semiquantitative determination of fecal urobilinogen is available It is carried out in an aqueous extract of fresh feces, and any urobilin present is reduced to urobilinogen by treatment with alkaline ferrous hydroxide before Ehrlich's reagent is added

- A range of 75-275 Ehrlich units/100 g of fresh feces or 75-400 Ehrlich units per 24-hour specimen is considered a normal reference range.

Measurement of Serum Bile Acids

- Unfortunately; complex methods are required for the analysis of bile acids in serum
- they involve extraction with organic solvents, partition chromatography; gas chromatography-mass spectroscopy spectrophotometry ultraviolet light absorption, fluorescence, radioimmunoassay and enzyme immunoassay methods
- Although serum bile acid levels are elevated in liver disease, the total concentration is extremely variable and adds no diagnostic value to other tests of liver function

= زيادة Bile acids بالدم :.

١. مشكلة بالـ Liver .

٢. مشكلة بالـ gall bladder .

Enzyme Tests in Liver Disease

- Any injury to the liver that results in cytolysis and necrosis causes the liberation of various enzymes.
- The most common enzymes assayed in hepatobiliary disease include ALP and the aminotransferases. (AST)
- Used less often are γ-glutamyltransferase, lactate dehydrogenase (LD) and its isoenzymes, 5'-nucleotidase, ornithine carbamoyltransferase, and leucine aminopeptidase

. test أقل استخدام لهل ⟸

= هاي الانزيمات ممكن تكون بكميات بسيطة جدًا بالدم نتيجة cells turnover . (طبيعي)

= اذا بك تيعم بكميات كبيرة معناها في damage واذا لفترات طويلة معناها damage مستمر.

Enzyme Tests in Liver Disease

(ALP)

Alkaline Phosphatase: in the clinical diagnosis of **bone** and **liver** disease.

- The most striking elevations occur in extrahepatic biliary obstruction, such as a stone in the common bile duct, or in intrahepatic cholestasis, such as drug cholestasis or primary biliary cirrhosis. This enzyme is almost always increased in metastatic liver disease and may be the only abnormality on routine liver function tests. in (Liver cancer)
- The enzyme is found in **placenta**, and **pregnant** women also have elevated levels and in children

Aminotransferases (Transaminases)

- AST and ALT are two enzymes widely used to assess hepatocellular damage. AST is found in all tissue, especially heart, liver, and skeletal muscle.
- ALT is more "liver specific"

واذا المشكلة بالكبدي biliary ALT مرتفع كتير.

- بيرتفع في ٥ حالات :

١. childhood ٢. pregnant women ٣. metastatic liver disease ٤. biliary obstruction ٥. intrahepatic cholestasis

Enzyme Tests in Liver Disease

diffrential enzyme.

5'-Nucleotidase: is another phosphatase in the liver and used clinically to determine whether an ALP elevation is caused by liver or bone disease

هاد بنرتق فيه في حالات بكون مرتفع ال ALP فبتأكد من: اذا كان 5NT كمان مرتفع معناها المشكلة بال Liver.

- This enzyme is much more sensitive to metastatic liver disease than is ALP because, unlike ALP, its level is **not** significantly elevated in other conditions, such as **pregnancy** or **childhood**

- Some increase in its activity may occur after **abdominal surgery**

برتفع في ٣ حالات:

1. abdominal surgery
2. metastatic liver disease
3. gall bladder obstruction

Enzyme Tests in Liver Disease

- **γ-Glutamyltransferase (GGT)**: high in kidney and the liver and is elevated in the serum of almost all patients with hepatobiliary disorders
  - It is not specific for any type of liver disease but is frequently the first abnormal liver function test demonstrated in the serum of persons who consume large amounts of alcohol
  - The highest levels are seen in biliary obstruction
  - Sensitive test for alcoholic liver disease
  - Measurement of this enzyme is also useful if jaundice is absent for the confirmation of hepatic neoplasms and to confirm hepatic disease in patients with elevated ALP

- **Leucine Aminopeptidase**: widely distributed in human tissue, is found in the pancreas, gastric mucosa, liver, spleen, brain, large and small intestine, and kidney.
  - The serum activity of leucine aminopeptidase cannot be used to differentiate hepatocellular from obstructive jaundice.
  - The measurement of this enzyme does not provide any useful information

(GGT) ⇒ very sensitive indicator for alcoholic liver damage.

Enzyme Tests in Liver Disease

- **Lactate Dehydrogenase**: Measurement of total serum LD is usually not helpful diagnostically because LD is present in all organs and released into the serum from various tissue injuries

- Fractionation of LD into its five tissue-specific isoenzymes may give useful information about the site of origin of the LD elevation

- LD-5 is mostly present in liver and skeletal muscle. elevated LD-5 is noted in a patient with jaundice

- Moderate elevations of total serum LD levels are common in acute viral hepatitis and in cirrhosis, whereas biliary tract disease may produce only slight elevations

- High serum levels may be found in metastatic carcinoma of the liver.

Tests Measuring Hepatic Synthetic Ability

- The measurement of the end products of hepatic synthetic activity can be used to assess liver disease. Although these tests are not sensitive to minimal liver damage, they are useful in quantitating the severity of hepatic dysfunction

- Most serum proteins are produced by the liver. A decreased serum **albumin** may be a result of decreased liver protein synthesis. The albumin level correlates well with the severity of functional impairment and is found more often in chronic rather than acute liver disease. The serum **α-globulins** (α1-antitrypsin) tend to decrease with chronic liver disease

- Serum **γ-globulin** levels are transiently increased in acute liver disease and remain **elevated in chronic liver disease.** The highest elevations are found in **chronic active hepatitis** and postnecrotic cirrhosis.

- IgG and IgM levels are more consistently elevated in chronic active hepatitis, IgM in primary biliary cirrhosis, and IgA in alcoholic cirrhosis.

γ-globulins ⇒ immunoglobulins that elevated when inflammation is present

Tests Measuring Hepatic Synthetic Ability

Prothrombin time : the time needed to form Clots of blood .

- **Prothrombin time** is commonly increased in liver disease because the liver is unable to manufacture adequate amounts of clotting factor or because the disruption of bile flow results in inadequate absorption of vitamin K from the intestine

- Response of the prothrombin time to the administration of vitamin K is of some value in differentiating intrahepatic disease with decreased synthesizing capacity from extrahepatic obstruction with decreased absorption of fat-soluble vitamins.

- A marked prolongation of the prothrombin time indicates severe diffuse liver disease and a poor prognosis

Scanned with CamScanner

Tests Measuring Nitrogen Metabolism

- The liver plays a major role in removing ammonia from the bloodstream and converting it to urea so in liver failure ammonia will increase leading to coma ↑ ammonia ↑ glutamate .

urea ← ammonia تحول ⇐ مارح

فبتتراكم ammonia وبتغير pH الدم

وبتدخل عالـ brain .

- In brain, glutamate react with ammonia to give glutamine which increases in CSF to cause encephalopathy

هدول السلايدين حسيتها حكت بس
اللي بالسلايدات وما طلعت عنهم ف
احفظوهم نفس ما همه او اسمعو
بداية ريكورد محاضرة ١٥ اذا حابين

Hepatitis

- inflammation of the liver, may be caused by viruses, bacteria, parasites, radiation, drugs, chemicals, or toxins.
- Among the viruses causing hepatitis are hepatitis types A, B, C, D (or delta), and E, **cytomegalovirus**, **Epstein-Barr** virus, and probably several others.
- Hepatitis A is usually transmitted by the **fecal/oral** route and causes a mild or inapparent infection with no tendency to chronic disease.
- Hepatitis B and C are primarily transmitted **parenterally**. Hepatitis B causes a serious illness in a minority of patients, however, in many patients, the infection is mild or even inapparent

Hepatitis

- Acute infection with hepatitis C is usually mild to inapparent
- Hepatitis B has a slight tendency to chronic disease, while most patients with hepatitis C infection develop chronic infection.
- Delta hepatitis is a unique satellite virus that causes a superinfection in patients already infected with hepatitis B.
- Hepatitis E is primarily transmitted by the **fecal/oral** route and causes serious disease **only** in **pregnant** women
- Chronic hepatitis is a major cause of morbidity and mortality worldwide
- Chronic hepatitis is a major risk factor for the development of hepatocellular carcinoma