Liver Function Tests

The liver is of vital importance in intermediary metabolism and in the detoxification and elimination of toxic substances. Damage to the organ may not obviously affect its activity since the liver has considerable functional reserve and, as a consequence, simple tests of liver function (e.g., plasma bilirubin and albumin concentrations) are insensitive indicators of liver disease. Tests reflecting liver cell damage (particularly the measurement of the activities of hepatic enzymes in plasma) are often superior in this respect.

Serum bilirubin:

Objectives:

1. Outline the Patient Preparation, Specimen Collection and Storage, Reference Range for bilirubin analyses
2. Describe the principle of determination of bilirubin:
3. Calculate the concentration serum bilirubin from reading the absorbance of standard and sample against their blank

Bilirubin is derived mainly from the haem moiety of haemoglobin molecules and is liberated when senescent red cells are removed from the circulation by the reticuloendothelial system; the iron in haem is reutilized but the tetrapyrrole ring is degraded to bilirubin.
Unconjugated bilirubin is not water-soluble; it is transported in the blood stream bound to albumin. In the liver it is taken up by hepatocytes where it undergoes conjugation, principally with glucuronic acid. Conjugated bilirubin is water soluble and is secreted into the biliary canaliculi, reaching the small intestine via the duct of the biliary system. In the gut, bilirubin is converted by bacterial action into urobilinogen, a colourless compound. Some urobilinogen is absorbed from the gut into the portal blood. Hepatic uptake of this is incomplete; a small quantity reaches the systemic circulation and is excreted in the urine. Most of the urobilinogen in the gut is oxidized in the colon to a brown pigment, stercobilin, which is excreted in the stool. The bilirubin normally present in the plasma is mainly unconjugated; since it is protein bound, it is not filtered by the renal glomeruli and, in health, bilirubin is not detectable in the urine. Hyperbilirubinemia can be caused by increase production of bilirubin, impaired metabolism, decrease excretion or a combination of these.

**Jaundice:**
Yellowish discoloration of skin, nail bed and sclera of the eye caused by deposition of bilirubin secondary to increase bilirubin level in the blood.

**Types of jaundice:**

1. **pre-hepatic (hemolytic):**
The liver has a capacity to conjugate and excrete over 3000mg of bilirubin per day, whereas the normal production is only 300mg/ day. Massive lysis of RBC e.g. (sickle cell anemia, pyruvate kinase deficiency, G6PD, malaria) may produce bilirubin faster than it can conjugated which lead to increase level of bilirubin excreted into the bile.

2. **hepatocellular:**
Damage to the liver cell e.g. patients with hepatitis or cirrhosis. Unconjugated bilirubin level increase due to decrease conjugation and regurgitation of conjugated bilirubin to the blood because conjugated bilirubin not efficiently excreted.

3. **post-hepatic (obstructive):**
Due to obstruction of bile duct e.g. hepatic tumor, bile stone. Patient with obstructive jaundice experience gastrointestinal pain, nausea with pale clay color stool. Conjugated bilirubin regurgitates from the liver which increase in its level.
Patient Preparation, Specimen Collection and Storage, Reference Range

1. **Patient Preparation**
   - AM fasting specimen preferred (avoid lipemia) [for total and direct bilirubin]

2. **Specimen collection**
   - serum or heparinized plasma (no hemolysis or lipemia) [for total and direct bilirubin]
   - urine: fresh random specimen preferred [for total and direct bilirubin]

3. **Specimen storage**
   - serum/plasma: protect from exposure to light (bilirubin is photooxidized, causing unconjugated form to react with Diazo reagents as well as conjugated form)
   - stored at low temperature (minimized photooxidation)
   - stable 3 days at 1-6°C, 3 months at -70°C
   - urine: protect from light to avoid oxidation. Stable 1 day at 1-6°C.

Reference range:
- serum/plasma [for total bilirubin] < 1.5 mg/dl
- serum/plasma [for direct bilirubin] < 0.2 mg/dl
- urine (random): negative.

**Determination of bilirubin:**

**Principle:**
Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of Dimethyl sulfoxide, total bilirubin reacts with diazotized sulfanilic acid to form azobilirubin. In the absence of Dimethyl sulfoxide only direct bilirubin reacts with diazotized sulfanilic acid to form azobilirubin.

**Procedure:**
**Total bilirubin:**

<table>
<thead>
<tr>
<th>Standard</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blank</td>
</tr>
<tr>
<td>Standard R4</td>
<td>50 μl</td>
</tr>
<tr>
<td>Sample</td>
<td>—</td>
</tr>
<tr>
<td>Reagent R1</td>
<td>1 ml</td>
</tr>
<tr>
<td>Working solution</td>
<td>—</td>
</tr>
</tbody>
</table>

**Wavelength:** 555nm (Hg 546)
**Cuvette:** 1 cm light path
**Temperature:** 37°C
**Zero adjustment:** Reagent blank

Mix well and incubate exactly 5 minutes at 37°C.
Read the absorbance of standard and sample against their blank.
Study questions:

Q1  Choose the most appropriate answer
1. Which enzyme is responsible for the conjugation of bilirubin?
   a. β-Glucuronidase
   b. **Uridine dihydrogen phosphate (UDP)-glucuronyl transferase**
   c. Bilirubin oxidase
   d. Biliverdin reductase
2. Which condition is caused by deficient secretion of bilirubin into the bile canaliculi?
   a. Gilbert's disease
   b. Neonatal hyperbilirubinemia
   c. Dubin-Johnson syndrome
   d. Crigler-Najjar syndrome
3. Which statement regarding total and direct bilirubin levels is true?
   a. **Total; bilirubin level is less sensitive and specific marker of liver disease than the direct level**
   b. Direct bilirubin level exceeds 3.5 mg/dL in most cases of hemolytic anemia
   c. Direct bilirubin is normal in cholestatic liver disease
   d. The ratio of direct to total bilirubin exceeds 0.4 in hemolytic anemia.
4. Which statement about colorimetric bilirubin methods is true?
   a. Direct bilirubin must react with diazo reagent under alkaline conditions
   **b. Most methods are based upon reaction with diazotized sulfanilic acid**
   c. Ascorbic acid can be used to eliminate interference caused by Hgb
   d. The color of the azobilirubin product independent of pH.
Transaminase (Aminotransferase) Enzymes

Objectives:
1. Outline the Patient Preparation, Specimen Collection and Storage, Reference Range for transaminase analyses
2. Describe the principle of determination of transaminases (ALT and AST):
3. State the diagnostic significance of transaminases:
4. Calculate the concentration serum transaminases from reading the absorbance of standard and sample against their blank

- The function of these enzymes is a transfer of an amino group from α-amino acid to α-keto acid in a reversible reaction with pyridoxal-5'-phosphate as a coenzyme.

\[
\text{L-Aspartate} + \text{2-Oxoglutarate} \xleftrightarrow{\text{AST, P-5'-P}} \text{Oxaloacetate} + \text{L-Glutamate}
\]

\[
\text{L-Alanine} + \text{2-Oxoglutarate} \xleftrightarrow{\text{ALT, P-5'-P}} \text{Pyruvate} + \text{L-Glutamate}
\]
The transamination reaction is important in the synthesis and degradation of amino acids. The keto acids formed are ultimately oxidized by TCA cycle to provide energy.

- Amino transferases act by transferring amino-group of an amino acid to the pyridoxal part of co-enzyme to form pyridoxamine phosphate, the pyridoxamine form of co-enzyme then react with α-ketoacid to form amino acid.
- The clinical importance of measuring these enzymes is to assess organ function and/or tissue damage, because transaminase are normally intracellular enzyme with low level found in plasma representing the release of cellular contents during normal cell turn over, the presence of high plasma enzyme level indicate damage to cell rich in these enzyme.

**A) AST:** present in heart, liver, skeletal muscle and to a lesser extent kidney, pancreas and RBCs. N.R. is 5-30 U/L.

**Diagnostic significance:**
1. Acute MI: ↑ within 6-8 hours, peak at 12-24 hours and return normal within 5 days.
2. Hepatocellular damage: ↑ in relation to the extent of tissue damage;
   - Acute viral hepatitis → may increase up to 50 times ULN.
   - Toxic liver injury by drugs or toxins, AST has an important role in assessing alcoholic hepatitis.
   - Cirrhosis → increase slightly to 4 times ULN.
3. Skeletal muscle disease: associated with an increase in CK enzyme level, it may even be affected by IM injections.
4. Hemolytic anemia: the enzyme is released from RBCs.

- Haemolyzed specimens are not suitable for analysis; plasma is stable for only 3-4 days in refrigerator.
- There are two isoenzymes of AST: cytoplasmic and mitochondrial, normally cytoplasmic fraction is more than mitochondrial fraction but in case of liver injury mitochondrial fraction predominates. AST isoenzyme fractionation is not routinely done in clinical labs.

**B) ALT:** present mainly in liver and to a lesser extent in kidney, skeletal muscle and pancreas. N.R. is 6-35 U/L.

**Diagnostic significance:**
1. Acute hepatitis: it is more important than AST because it appears earlier (may be before jaundice), with a higher peak & remains longer due to longer half-life (t½ of AST= 16 hours, t½ of ALT=24 hours).
2. Liver cirrhosis.
3. MI if associated with hepatic congestion. Measuring ALT in these conditions help to exclude the diagnosis of MI, when both enzymes are increased it indicates a liver source, but if only AST is increased this indicates a cardiac source.
4. Toxic liver injury or even a drug abuse of salicylates or narcotics.

- Enzymes are usually assessed by measuring their activity using Activity Units (IU).
- **IU** is the amount of enzyme that will catalyze the reaction of 1 μmol of substrate per minute under specified conditions of temperature, pH, substrates, and activators.
- Enzyme concentration is measured as (IU/L).
Principle of Transaminase measurement:
Colorimetric determination of AST or ALT activity according to the following equations:

\[
\text{Aspartic acid} + \alpha\text{-Ketoglutaric acid} \xrightarrow{\text{AST}} \text{Oxaloacetic acid} + \text{Glutamic acid} \\
\text{(Asp.)} \quad \text{(\(\alpha\text{-KG}\))} \quad \text{PLP} \quad \text{(Oxa.)} \quad \text{(Glu.)}
\]

\[
\text{Alanine} + \alpha\text{-Ketoglutaric acid} \xrightarrow{\text{AST}} \text{Pyruvic acid} + \text{Glutamic acid} \\
\text{(Ala.)} \quad \text{(\(\alpha\text{-KG}\))} \quad \text{PLP} \quad \text{(Pyr.)} \quad \text{(Glu.)}
\]

The oxaloacetate or pyruvate formed is measured in its derivative form 2,4-dinitrophenylhydrazone (DNPH).

Measurement against reagent blank:

<table>
<thead>
<tr>
<th></th>
<th>Serum sample</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer reagent pH 7.4</td>
<td>0.1 ml</td>
<td>−</td>
</tr>
<tr>
<td>Reagent 2</td>
<td>0.5ml</td>
<td>0.5ml</td>
</tr>
<tr>
<td>Mix. incubate for <strong>exactly 30 minutes</strong> at 37°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,4-DNPH</td>
<td>0.5ml</td>
<td>0.5ml</td>
</tr>
<tr>
<td>Serum</td>
<td>−</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Mix. allow to stand for 20 min. at 37°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaOH</td>
<td>5.0ml</td>
<td>5.0ml</td>
</tr>
</tbody>
</table>

Mix, read the absorbance of the sample against the reagent blank after 5 minutes.

Study questions:

Q1. Choose the most appropriate answer

1. Which of the following statement about the amino-transferases (AST and ALT) is true?
   a. Isoenzymes of AST and ALT are not found in humans
   b. **Both transfer an amino group to \(\alpha\text{-ketoglutarate.}\)**
   c. Both require NADP+ as a coenzyme
   d. Both utilize four carbon amino acids as substrates.

2. Select the products formed from the forward reaction of AST:
   a. Alanine and \(\alpha\text{-ketoglutarate}\)
   b. **Oxaloacetate and glutamate**
   c. Aspartate and glutamate
   d. Glutamate and NADH

3. Select the products formed from the forward reaction of ALT:
   a. Aspartate and alanine
   b. Alanine and \(\alpha\text{-ketoglutarate}\)
   c. **Pyruvate and glutamate**
   d. Glutamate and NADH
4. Which condition gives rise to the highest serum level of transaminase?
   a. Acute hepatitis
   b. Alcoholic cirrhosis
   c. Obstructive biliary disease
   d. Diffuse intrahepatic cholestasis

5. In which liver disease is the De Ritis ratio (ALT:AST) usually greater than 1.0?
   a. Acute hepatitis
   b. Chronic hepatitis
   c. Hepatic cirrhosis
   d. Hepatic carcinoma