

ESTIMATION OF SERUM BILIRUBIN LEVELS AND RELATED CLINICAL CASES

AIM- To estimate serum bilirubin level.

INTRODUCTION- Bilirubin is derived by breakdown of heme (part of hemoglobin, myoglobin, cytochromes, catalase, peroxidase, etc.). Hemoglobin present in RBCs is the major source of bilirubin. Heme catabolism occurs in reticuloendothelial system (spleen and liver) and the bilirubin formed is unconjugated. Unconjugated bilirubin is insoluble in water. It is transported in blood bound to albumin and is taken up by hepatocytes to convert unconjugated bilirubin to conjugated bilirubin. This conjugated form is water soluble and is secreted in bile. Bile is stored in gall bladder from where it passes into duodenum via cystic and common bile duct. In the intestine, it is reduced by bacteria to urobilinogen, which is reabsorbed from large intestine. Most of it is secreted in bile by liver again via enterohepatic circulation. A small part is excreted in stool (urobilinogen to stercobilinogen to stercobilin) and urine.

NORMAL LEVELS IN SERUM- Total bilirubin-0.2-1.0 mg/dl

Conjugated bilirubin-0.1-0.4 mg/dl

Unconjugated bilirubin-0.2-0.7 mg/dl

ESTIMATION- MALLOY AND EVELYN METHOD

PRINCIPLE- This method for bilirubin estimation is based on Van Den Bergh reaction. In this reaction, bilirubin reacts with diazotized sulfanilic acid to produce azobilirubin which is purple in color. Intensity of color is directly proportional to the amount of bilirubin in the serum.

There are two kinds of reactions- direct reaction and indirect reaction.

The **direct reaction** occurs in aqueous medium and is given by **only water-soluble conjugated bilirubin**. The reaction is fast and color develops in first few minutes only. That is why, conjugated bilirubin is also called direct bilirubin.

In indirect reaction, methyl alcohol is added which solubilizes unconjugated, water insoluble bilirubin. Hence, **indirect reaction gives the measure of total bilirubin (conjugated and unconjugated)**.

Unconjugated bilirubin = total bilirubin- conjugated bilirubin

In this way, unconjugated bilirubin can be calculated after indirect reaction. Also, unconjugated bilirubin is called indirect bilirubin.

REAGENTS

- Diazo reagent: sulfanilic acid and sodium nitrate in HCl
- Diazo blank
- Methyl alcohol
- Standard: Known concentration

CALCULATION (WILL BE DISCUSSED AGAIN DURING PRACTICAL SESSION)

Conc. of bilirubin (serum sample/test) = $\text{OD}(\text{test})/\text{OD}(\text{standard}) * \text{conc. of standard} * 100$
mg/dl

Calculation is followed by result and interpretation on the basis on Serum total bilirubin, conjugated (direct) bilirubin and unconjugated (indirect) bilirubin.

ABNORMAL FINDINGS IN BLOOD/SERUM, URINE AND STOOL IN DIFFERENT TYPES OF JAUNDICE

	PRE-HEPATIC	HEPATIC	POST-HEPATIC
BLOOD			
Total bilirubin	++	++	++
Unconjugated (indirect) bilirubin	+++	++	+
Conjugated (direct) bilirubin	+	++	+++
URINE			
Urobilinogen	++	+	-
Bile salts	-	+/-	++
Bilirubin	-	+/-	++
STOOL COLOR	Dark	normal	Clay

POINTS TO PONDER: WHAT ARE THE ENZYMATIC MARKERS FOR PRE-HEPATIC, HEPATIC AND POT HEPATIC JAUNDICE?

CLINICAL CASES: PROBLEM BASED LEARNING

INSTRUCTIONS: PREPARE THE ANSWERS TO THE QUESTIONS FOR THE FOLLOWING CASES

Case1.

A 35 years obese woman presented in medical OPD with complaints of mild fever, loss of appetite and pruritis (itching). She gave history of an acute attack of pain in right hypochondrium with vomiting previous day. There was history of passing frothy, clay colored stool and high colored urine. There was no clear-cut yellowish discoloration in the eyes. Results of Liver Function Test were:

Total bilirubin-5.0 mg/dl
 Direct bilirubin-3.8 mg/dl
 ALT- 60 IU/L
 AST- 50 IU/L
 ALP- 2000 IU/L

Questions:

1. What is your diagnosis and what could be the causes?

2. Why is the urine high colored?
3. What could be the findings in the urine of this patient (bilirubin, urobilinogen, bile salts)?
4. What is the cause of clay colored stool?
5. Why are enzymes raised (especially ALP)?

Case2.

A 14 years old girl was admitted to the medical ward after she developed yellowish discoloration of the eye, marked loss of appetite, low grade fever, nausea and occasional vomiting in the last one week. She had pain in right hypochondrium and urine was high colored. The stool was clay colored. She looked weak and malnourished also. Results of Liver Function Test were:

Total bilirubin-8.0 mg/dl
Direct bilirubin-4.8 mg/dl
ALT- 980 IU/L
AST- 1210 IU/L
ALP- 500 IU/L

Questions:

1. What is the type of jaundice in this patient? Explain the causes.
2. How the diagnosis of viral hepatitis is confirmed? Which types are associated with high morbidity and mortality?
3. Which type of bilirubin is increased in blood?
4. Why there is pain in the right hypochondrium, yellowish discoloration of eyes and clay colored stool?
5. Which test would you do to detect bile pigments in the urine sample of this patient?

Case3.

A new born preterm baby developed jaundice on 3rd day which first increased moderately and then gradually subsided. The child was exposed to blue light as a part of treatment. Serial blood investigations revealed following results:

1st day Serum total bilirubin-2.5 mg/dl
Serum direct bilirubin-0.6 mg/dl

3rd day Serum total bilirubin- 8.0mg/dl
Serum direct bilirubin-2.5 mg/dl

5th day Serum total bilirubin-6.5 mg/dl
Serum direct bilirubin-3.0 mg/dl

7th day Serum total bilirubin-2.0 mg/dl
Serum direct bilirubin-1.0 mg/dl

Questions:

1. What is the probable diagnosis and the cause?
2. What is the role of blue light (phototherapy)?
3. What is the difference between neonatal physiological jaundice and pathological jaundice?
4. What is the consequence of persistent unconjugated hyperbilirubinaemia?