Measurement of Bilirubin

(Test of excretory function of liver)

Bilirubin is the excretory product formed by the catabolism of heme. It is conjugated by the liver to form bilirubin diglucuronide and excreted through bile.

Measurements of bilirubin as well as detection of bilirubin and urobilinogen in urine are important tests of liver function.

Normal serum bilirubin level varies from **0.2 to 0.8 mg/dl**

The unconjugated bilirubin (bilirubin-albumin complex) (free bilirubin) (indirect bilirubin) varies from 0.2–0.7 mg/dl and conjugated bilirubin (direct bilirubin) 0.1–0.4 mg/dl.

A rise in serum bilirubin above 1 mg/dl is abnormal (latent jaundice); but jaundice appears only if the level goes above 2 mg/dl.

The bilirubin is estimated by **van den Bergh reaction,**. Normal serum does not give a positive van den Bergh reaction.

When bilirubin is **conjugated**, the purple color is produced immediately on mixing with the reagent, the response is said to be van den Bergh **direct positive**.

When the bilirubin is **unconjugated**, the color is obtained only when alcohol is added, and this response is known as **indirect positive**.

If both conjugated and unconjugated bilirubins are present in increased amounts, a purple color is produced immediately and the color is intensified on adding alcohol. Then the reaction is called **biphasic**.

In **hemolytic** jaundice, unconjugated bilirubin is increased. Hence van den Bergh test is indirect positive. In **obstructive** jaundice, conjugated bilirubin is elevated, and van den Bergh test is direct positive. In **hepatocellular** jaundice, a biphasic reaction is observed, because both conjugated and unconjugated bilirubins are increased.

Urinary Bilirubin

i. In all cases of jaundice, urine should be examined for the presence of bile pigments (bilirubin), bile salts and urobilinogen.

ii. Only conjugated bilirubin is soluble in water and is excreted in urine. Hence in prehepatic jaundice, when the unconjugated bilirubin is increased in blood, it does not appear in urine.

iii. But in obstructive jaundice, conjugation of bilirubin is taking place, which cannot be excreted through the normal passage, and so it is regurgitated back into bloodstream; this is then excreted through urine. So in obstructive jaundice, urine contains bilirubin.

3. Urinary Urobilinogen

i. In cases of obstruction, bile is not reaching the intestine and so urobilinogen may be decreased or absent in urine.

ii. In hepatocellular jaundice, urobilinogen is initially elevated **(why?)** then decreases or disappears when the obstructive stage sets in and reappears when obstruction is cleared.

iii. Urobilinogen is absent in urine, when there is obstruction to bile flow. The first indication of the recovery is the reappearance of urobilinogen in urine.

iv. In hemolytic anemias, urobilinogen is increased.

Bilirubin is detected by Fouchet's test and urobilinogen by Ehrlich's test. The findings in urine in different types of jaundice are shown in table.

Relate with this the hay’s test (next slide)

Under stand the classification and causes for jaundice.

Table that gives the tests to distinguish different types of jaundice.

Urine Bile Salts

Normally bile salts (sodium salts of taurocholic acid and glycocholic acid) are present in the bile; but are not seen in urine. Bile salts in urine are detected by **Hay's test**. Positive Hay's test indicates the obstruction in the biliary passages causing regurgitation of bile salts into the systemic circulation leading to its excretion in urine.

Obstruction can occur in obstructive jaundice and also in hepatic jaundice due to obstruction of micro biliary channels caused by inflammation.

Tests		Jaundice type		
		Pre Hepatic	Intra Hepatic	Post Hepatic
Serum	Conj. Blrb	N (saturation)	E (cell lysis)	E(regurg)
	Unconj. Blrb	E (high RBC lysis)	E (cell lysis)	N
	Bile salts	N	E(cell lysis)	E(regurg)
	AST/ALT	N	E(cell lysis)	N
	GGT	N	N	E(regional)
	5'NT	N	N	E(regional)
	Alk P	N	N	E(regional)
	vanden	indirect	biphas	direct

tests		Jaundice type		
		Pre Hepatic	Intra Hepatic	Post Hepatic
urine	Conj. Blrb	N	E(lysis)	E(regurg)
	Unconj. Blrb	-	-	-
	bilesalt		E(lysis)	E(regurg)
	UBG/ SBG	E(High production)	-	Nil
	vanden	??	??	direct