Bilirubin Secretion, Jaundice and Evaluation of Liver Function

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Evaluation of Liver Disease and Hepatic Function

History
Physical Examination
Laboratory Tests
Sometimes Radiological/Nuclear Medicine
Sometimes Liver Biopsy
Jaundice occurs as a result of excess bilirubin in the blood. It is a hallmark of liver disease but not always present in liver disease. Jaundice occurs when the liver fails to adequately secrete bilirubin from the blood into the bile. To understand how jaundice occurs, you must first understand bilirubin synthesis, metabolism and secretion.
NET MOVEMENT FROM BLOOD TO BILE INVOLVES MULTIPLE STEPS

DELIVERY
Sinusoid
Hepatocyte

UPTAKE
“STORAGE”

BIOTRANSFORMATION

REFLUX
Canaliculus

SECRETION
Bile Duct

BILIARY FLOW

Lipophilic compound
Polar metabolite
BILIRUBIN IS PRODUCED BY
OXIDATION OF HEME AND
REDUCTION OF THE RESULTANT BILIVERDIN

HEME

BILIVERDIN IX α

M = Methyl
V = Vinyl
Pr = Propionyl

O₂ & NADPH
Heme Oxygenase

CO

Fe³⁺

NADP⁺

BILIRUBIN IX α

NADP⁺

Biliverdin Reductase

NADPH
Heme Oxygenase
Schuller et al. 

Bilivirdin Reductase
Kikuchi et al. 
*Nature Structural Biology* 8, 221 - 225 (2001)
THERE ARE MULTIPLE SOURCES OF BILIRUBIN

2-{superscript 14}C-glycine → δ-Aminolevulinate → Porphobilinogen → Protoporphyrin → Biliverdin → Bilirubin

Bilirubin Specific Activity

Early Peak
- Ineffective Erythropoiesis (Bone Marrow)

Hepatic Heme Turnover

Late Peak
- Destruction of Senescent Red Cells (Spleen)

80%

Days after {superscript 14}C-glycine Administration
Bilirubin is frequently depicted as a linear tetrapyrrole. However, intramolecular hydrogen bonding fixes it in a rigid structure that blocks exposure of its polar groups to aqueous solvents, making it very insoluble in blood.
Bilirubin in Blood is Bound to Albumin: Uptake into Hepatocyte at Basolateral (Sinusoidal) Membrane

Some bilirubin stored in cytosol bound to proteins
Bilirubin UDP-glucuronosyltransferase is localized to the endoplasmic reticulum; it catalyzes conjugation to a diglucuronide, making it more water soluble.

A: Labeling of periphery of cell hepatocyte nucleus
B: Labeling of ER with antibody to UDP-glucuronosyltransferase
Alternative RNA splicing of different first exons of \textit{UGT1} gives different isoforms with different substrate specificities, some for bilirubin and others to different substrates, such as phenol.
BILIRUBIN CONJUGATION IN MICROSONES INVOLVES TWO STEPS, MEDIATED BY ONE BILIRUBIN-UDPGA TRANSFERERASE

BILIRUBINS:

UNCONJUGATED (UCB) → MONO-GLUCURONIDE (BMG) → DIGLUCURONIDE (BDG)

Conjugation prevents internal H-bonding by the -COOH groups of Bilirubin
Bilirubin glucuronide is secreted from hepatocytes by an ATP-binding cassette protein. This is the rate-limiting step in hepatocellular bilirubin metabolism and disrupted in most acquired liver diseases.
Bilirubin is Only Approximately 2% of Bile
Some urobilinogen excreted by kidney

STERCOBILINS/UBROBILINS IN STOOL
Diagnostic Consequences of Enterohepatic Circulation of Bilirubin

• In hepatocyte dysfunction (hepatocellular)
  – May see increased urobilinogen in urine because it is less efficiently reabsorbed by hepatocytes

• In biliary obstruction
  – Stools may appear white because bilirubin does not get into intestine and therefore not converted to stercobilins/urobilins
  – No urobilinogen detected in urine
Measurement of Bilirubin in Blood

• Normally ≤17 µM (1 mg/dl)
  – >35 µM: can begin to detect jaundice clinically, (sclera, mucus membranes early)
  – Discoloration of skin with higher concentrations
• When measured precisely (e. g. by HPLC), around 96% of serum bilirubin is unconjugated
• Clinical laboratory generally “overestimates” amount of conjugated bilirubin (up to 30%) because of method
  – reported as “total,” “direct” (approximates conjugated) and “indirect” (approximates unconjugated)
van den Bergh and Muller Reaction (1916)

Using this method, 20% to 30% serum direct bilirubin is normal value.
Excess conjugated bilirubin in serum may be excreted by kidneys (dark urine). Albumin-bound unconjugated bilirubin cannot be excreted by kidneys.

With longstanding elevated serum conjugated bilirubin, less is in urine because of covalent binding to albumin.
Causes of Hyperbilirubinemia and Jaundice
DISORDERS CAUSING UNCONJUGATED HYPERBILIRUBINEMIA

Overproduction

Impaired Uptake

Impaired Conjugation
UNCONJUGATED HYPERBILIRUBINEMIA CAUSED BY OVERPRODUCTION OF BILIRUBIN

**Hemolysis**

Intravascular—hemolytic anemia, transfusion reactions
Extravascular—resorption of hematoma

**Ineffective Erythropoiesis**

Megaloblastic anemia, Thalassemias

**Impaired Uptake**

Fasting, Sepsis, Gilbert syndrome?, Some drugs (e.g., probenecid)
UNCONJUGATED HYPERBILIRUBINEMIA CAUSED BY IMPAIRED BILIRUBIN CONJUGATION

Crigler-Najjar Syndrome Type I
Crigler-Najjar Syndrome Type II
Gilbert Syndrome
CRIGLER-NAJJAR SYNDROME

- Autosomal recessive inheritance
- Mutations in UGT1 gene resulting in decreased to absent bilirubin conjugation
- Type 1: absent activity
- Type 2: decreased activity with serum bilirubin concentrations from 8 to 20 mg/dl
- Animal model: Gunn rat
Most Common Cause of Unconjugated Hyperbilirubinemia Western Countries

GILBERT SYNDROME

- Mildly decreased UGT1 activity
- Mutations in the UGT1 gene promoter have been described
- Unconjugated serum bilirubin can range from 1.5 mg/dl to 6.0 mg/dl
- Other defects besides decreased conjugation such as decreased uptake may contribute
- Exacerbated by stress, fasting, and infection
- Serum bilirubin concentrations decreased by phenobarbital
- Fairly common and familial tendency

Gilbert syndrome is not really a “disease” but a normal variant.
"PHYSIOLOGICAL" NEONATAL JAUNDICE RESULTS FROM IMMATURITY OF ALL STEPS IN BILIRUBIN METABOLISM

1. Production
2. Delivery
3. Uptake
4. Ligandin
5. Conjugation
6. Reflux
7. Secretion
8. Biliary Flow

R-E System
Plasma
Canaliculus
Bile Duct
Hepatocyte
High blood concentrations of lipid soluble unconjugated bilirubin in infants that also have poorly developed blood-brain barrier can lead to kernicterus (brain damage caused by bilirubin deposition). Treatments include exchange transfusion and phototherapy. Heme oxygenase inhibitors are also being studied for this indication.
Less intramolecular hydrogen bonding of E diasteriomers make them more aqueous soluble for renal excretion.
DISORDERS CAUSING PRIMARILY CONJUGATED HYPERBILIRUBINEMIA

Impaired Secretion of Conjugated Bilirubin

Intrahepatic and Extrahepatic Biliary Tree Obstruction/Cholestasis
PRIMARILY CONJUGATED HYPERBILIRUBINEMIA CAUSED BY IMPAIRED SECRETION OF BILIRUBIN

Hepatocellular Diseases
Viral, drug, and alcoholic hepatitis, various metabolic diseases, cirrhosis

Pregnancy
Presumably related to estrogen sensitivity, similar to jaundice induced by birth control pills

Inherited Disorders
Dubin-Johnson syndrome, Rotor syndrome

Caused by mutation in ABCC2 encoding canalicular transporter.
PRIMARILY CONJUGATED HYPERBILIRUBINEMIA CAUSED BY BILIARY TREE OBSTRUCTION/CHOLESTASIS

- Strictures
- Gallstones
- Tumor
- Primary Biliary Cirrhosis
- Primary Sclerosing Cholangitis
- Biliary Cysts
- Drugs
- Others
With abnormal secretion from hepatocytes, most excess bilirubin in blood is conjugated by can get “mixed picture” because of “backup” of unconjugated bilirubin.
Laboratory Tests in Liver Diseases
Bilirubin

- From breakdown of heme (mostly from old red blood cells)
- Hyperbilirubinemia can occur in liver disease
- Not specific for liver disease – increased production (e.g. hemolysis)
Albumin

- Synthesized in hepatocytes and secreted into blood
- Half-life about 20 days
- Hypoalbuminemia can occur with hepatic synthetic dysfunction in chronic liver disease, especially advanced cirrhosis
- Not specific for liver disease (renal, cardiac, gut)
- Usually measured in g/dl
Prothrombin Time

- Reflects a type of blood clotting
- Several blood clotting factors are synthesized in the liver, most with half-lives of 9 to 26 hr
- Prothrombin time can be prolonged within a day in severe acute liver disease
- Also prolonged in severe chronic liver disease
- Not specific for liver disease
- Usually measured in seconds or INR
Glucose

- Glycogenolysis and gluconeogenesis take place in the liver
- In severe liver dysfunction, hypoglycemia can occur
- Cirrhosis sometimes associated with insulin resistance and elevated serum glucose
- Usually measured in mg/dL
Cholesterol

- Synthesized in the liver and secreted complexed as lipoproteins
- Serum cholesterol concentration will be low in severe hepatocellular dysfunction
- Biliary obstruction can lead to elevations in serum cholesterol, which will be part of an abnormal lipoprotein called lipoprotein X
- Usually measured in mg/dL
LIVER ENZYMES ARE NOT LIVER FUNCTION TESTS!

In chronic hepatitis, there is poor correlation between the magnitude of the liver enzyme elevations and the degree of liver injury.
Alanine aminotransferase (ALT)

- Present in cytosol of hepatocytes
- Catalyzes transamination between amino and \( \alpha \)-keto acids
- Serum activity is elevated when hepatocytes are damaged or destroyed (e.g. hepatitis, hepatic necrosis)
- Fairly specific for liver disease
- Measured in IU/L (activity)
Aspartate aminotransferase (AST)

- Present in cytosol and mitochondria of hepatocytes and muscle cells
- Catalyzes transamination between amino and α-keto acids
- Serum activity is elevated when hepatocytes are damaged or destroyed (e.g. hepatitis, hepatic necrosis)
- Also elevated in muscle disease and acute MI
- Measured in IU/L (activity)
Alkaline Phosphatase

- Present predominantly near the microvilli of the bile canaliculi
- Serum activity is elevated with intrahepatic cholestasis, extrahepatic biliary obstruction, or invasion of the liver (e.g., tumor, mycobacterial infections, etc.)
- Also present in bone and placenta and serum activity may be elevated in bone diseases
- Measured in IU/L (activity)
**γ-glutamyltranspeptidase**

- Present predominantly near the microvilli of the bile canaliculi
- Serum activity is elevated in same conditions as those that increase alkaline phosphatase
- Also induced by alcohol and other drugs that may cause serum elevations in activity
- Helpful to differentiate biliary disease from bone disease when serum alkaline phosphatase activity is elevated
- Measured in IU/L (activity)
Radiological/Nuclear Medicine

- Ultrasound
- Computerized Tomography
- Magnetic Resonance Imaging
- Liver–Spleen Scan
- Endoscopic Retrograde Cholangiopancreatography
- Tagged Red Blood Cell Scan
- Oral and Transhepatic Cholecystography
- Radionuclide Biliary Scans
Liver Biopsy in the Evaluation of Liver Disease

Dr. Lefkowitch Next