HEREDITARY HYPERBILIRUBINEMIAS: DIAGNOSTIC APPROACH AND MANAGEMENT. LECTURE FOR POSTGRADUATE MEDICAL STUDENTS

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Резюме. Hyperbilirubinemia appears as jaundice or icterus, which can usually be detected when the serum bilirubin level exceeds 34 μmol/L. There are different types of predominantly unconjugated and predominantly conjugated bilirubinemias. Diagnostic approach to patients with icterus or hyperbilirubinemia was proposed. Clinical presentation, diagnostic criteria and principles of treatment are described for Gilbert syndrome, Crigler-Najjar syndrome, Dubin-Johnson Syndrome and Rotor syndrome.

Conclusion: benign hyperbilirubinemias are rare syndromes but can be distinguished from other liver injures with difficulties.

Keywords: benign hyperbilirubinemias, Gilbert syndrome, Crigler-Najjar syndrome, Dubin-Johnson Syndrome and Rotor syndrome.

Hyperbilirubinemia appears as jaundice or icterus. Jaundice can usually be detected when the serum bilirubin level exceeds 34 — 43 μmol/L (2-2.5 mg/dL), or about twice the upper limit of the normal range, but may be detectable at lower bilirubin levels in patients with fair skin and profound anemia. Scleral tissue is rich in elastin, which has a high affinity for bilirubin, so that scleral icterus is usually a more sensitive and early sign of hyperbilirubinemia than generalized jaundice.

Production and metabolism of bilirubin. Approximately 80% of circulating bilirubin is derived from senescent red blood cells. When circulating erythrocytes reach the end of their normal life span of approximately 120 days, they are destroyed by reticuloendothelial cells. Hemoglobin dissociated to heme and globin, oxidation of the heme generates biliverdin, which is then metabolized to bilirubin. Unconjugated bilirubin liberated into plasma is bound tightly, but nonconvalently to albumin. The kidneys do not filter unconjugated bilirubin due to its avid binding to albumin. For this reason, the presence of bilirubin in the urine indicates the presence of conjugated hyperbilirubinemia.

Certain organic anions, such as sulfonamides and salicylates, compete with bilirubin for binding sites on albumin. Bilirubin is present in body fluids (joint effusions, ascitic fluid, pleural effusions, cysts, cerebrospinal fluid) in proportion to their albumin content and is absent in true secretions such as tears, saliva and pancreatic juice.

Hepatic metabolism of bilirubin can be divided into three distinct phases:

1. hepatic uptake;
2. conjugation by bilirubin-uridine diphosphate glucuronyl transferase (UGT) that converts bilirubin to a mixture of monoglucuronides and diglucuronides (conjugated bilirubin);
3. excretion into bile by an ATP-dependent transporter.

After these three processes, conjugated bilirubin is going through the biliary ducts into the duodenum. It is metabolized by ileal and colonic bacteria to urobilinogen.

Normal serum values of total bilirubin typically are 3.4-17.1 μmol/L (0.2-1 mg/dL), of which no more than 3.4 μmol/L (0.2 mg/dL) is directly reacting (conjugated) bilirubin.

When chemical analysis (van den Bergh reaction) reveals 80 — 85% of the total serum bilirubin to be unconjugated, a patient is considered to have primarily unconjugated hyperbilirubinemia. A patient with more than 50% direct-reacting (conjugated) serum bilirubin is considered to have predominantly conjugated hyperbilirubinemia (table 1,2).

Diagnostic approach to patients with icterus or hyperbilirubinemia

4. Careful clinical history and physical examination
5. Laboratory tests:

Table 1. Predominantly unconjugated bilirubinemias

I. Overproduction of bilirubin	A. Hemolysis (intra- and extravascular, especially acute hemolytic crises) B. Ineffective erythropoeisis (a rare disorder known as primary shunt hyperbilirubinemia or idiopathic dyserythropoietic jaundice with production of early labeled bilirubin)
II. Decreased hepatic uptake	Prolonged fasting, sepsis, congestive heart failure, portosystemic shunts; drugs/contrasts: rifampicin, rifamycin, probenecid, flavaspidic acid, bunamiodyl (cholecystographic agent)
III. Decreased bilirubin conjugation due to abnormal activity of hepatic glucuronosyl-transferase	A. Hereditary UGT deficiency: 1. Gilbert syndrome (mild deficiency) 2. Crigler – Najjar type II (moderate deficiency) 3. Crigler – Najjar type I (absence of UGT)
	B. Neonatal jaundice, either physiologic or non-physiologic (transient UGT deficiency; increased intestinal absorption of unconjugated bilirubin)
	C. Acquired UGT deficiency: 4. Drug inhibition (e.g., chloramphenicol, pregnanediol) 5. Breast milk jaundice (inhibition by pregnanediol and fatty acids in breast milk) 6. Hepatocellular diseases (hepatitis, cirrhosis) 7. Hypothyroidism/hyperthyroidism
IV Other	Fasting hyperbilirubinemia (contributed to increased enterohepatic circulation of bilirubin and genetic factors)

Table 2. Predominantly conjugated bilirubinemias

I. Impaired hepatic excretion (infrahepatic defects)	
A. Familial or hereditary disorders	1. Dubin-Johnson syndrome 2. Rotor syndrome 3. Recurrent (benign) intrahepatic cholestasis 4. Cholestatic jaundice of pregnancy
B. Acquired disorders	1. Infectious inflammation: hepatitis caused by hepatitis A-E viruses, Epstein-Barr virus, cytomegalovirus; sepsis 2. Non-infectious inflammation: toxic liver injury, alcoholic hepatitis, drug toxicity, hemochromatosis, Wilson disease, autoimmune hepatitis 3. Other: ischemia, preeclampsia, Reye syndrome, total parenteral nutrition
II. Extrahepatic biliary obstruction (disturbed bile flow into the intestine)	
A. Damage to intra-hepatic bile ducts	Primary biliary cirrhosis, sclerosing cholangitis, graft-versus-host disease, veno-occlusive disease
B. Obstruction of extrahepatic bile ducts	Choledocholithiasis, bile duct tumors, extrinsic compression of the bile duct, developmental disorders of the bile ducts, postsurgical strictures, acute pancreatitis, AIDS cholangiopathy
C. Diffuse infiltrative diseases	Amyloidosis, sarcoidosis, Wegener granulomatosis, disseminated mycobacterial infections, lymphoma, diffuse malignancy
D. Drug-induced cholestasis	Chlorpromazine, erythromycin, estrogens, anabolic steroids, etc.

- CBC count, including reticulocyte count and blood smear to screen for hemolytic disorders
- Fractionated bilirubin (unconjugated or conjugated bilirubinema)
- Urine test for bilirubin and urobilinogen
- Serum aminotransferases (AST, ALT) to exclude cytolitic syndrome
- Serologic screen for hepatitis (anti-HCV, HBsAg or anti-HBeAg)
- Markers of cholestasis: γ-glutamyl trans-peptidase (to differentiate a hepatic source from bone or other causes) or alkaline phosphatase. If elevated or if an obstruction is suspected, images of the bile ducts should be obtained.
- Test for antimitochondrial antibodies when considering primary biliary cirrhosis. Antinuclear antibodies, smooth-muscle antibodies, and other serologic studies when considering autoimmune hepatitis.
- Iron and genetic studies when considering hemochromatosis. Copper studies when considering Wilson disease
- Blood alcohol or acetaminophen levels upon admission
- Bile investigation

3. Imaging studies
   - Abdominal ultrasound should be performed to exclude biliary obstruction and to evaluate the liver parenchyma
   - Abdominal CT scans or magnetic resonance imaging (MRI) – provide additional information about patients with abnormal ultrasound scans.
   - Endoscopic retrograde cholangiopancreatography (ERCP) if biliary obstruction is suspected. It is the investigation of choice to detect common bile duct stones and is useful for making a diagnosis of pancreatic cancer, primary sclerosing cholangitis or choledochal cysts.

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- Percutaneous transhepatic cholangiography (PTC) can be useful in cases in which ERCP has been unsuccessful.

Hereditary glucuronyl transferase deficiencies. Gilbert syndrome

This syndrome was first reported by Augustine Gilbert and Pierre Lereboullet in 1901. It is autosomal recessive condition, characterized by mild, persistent, unconjugated hyperbilirubinemia with considerable daily and seasonal variations and intermittent jaundice in the absence of hemolysis or underlying liver disease.

The total serum bilirubin level usually fluctuates from 21 to 51 µmol/L (1.2 — 3 mg/dL) and rarely exceeds 86 µmol/L (5 mg/dL). With the van den Bergh diazoreaction, less than 20% of bilirubin gives a direct reaction. However, studies using more accurate methods (high-pressure liquid chromatography) show that almost all the serum bilirubin in patients with Gilbert syndrome is unconjugated.

Genetic data indicates that impaired bilirubin glucuronidation in patients with Gilbert syndrome may be caused by multiple mutations in exon 1a, its promoter, or other exons of *UGT1* gene that is located on chromosome 2 and encodes bilirubin-uridine diphosphate glucuronyl transferase (UGT). Most patients have reduced expression of the *UGT1* gene due to the presence of two extra nucleotides in the promoter region of the gene (TATAA promoter region), resulting in a partial deficiency of UGT. Their hepatic glucuronidating activity is approximately 30% of normal.

Incidence. Gilbert syndrome affects 4-13% of the population. It is not restricted to any ethnic group and occurs in persons of all races; however genetic studies have shown that mutations in the TATAA promoter region affect 36% of Africans, but only 3% of Asians. The clinical phenotype may not be as apparent as the determined genotype because of environmental influences which can reduce bilirubin levels.

The patient usually does not manifest this disorder until after the second decade. It occurs predominately in men (a male-to-female ratio 2-7:1).

Signs and symptoms

1. Jaundice. It fluctuates and often follows surgery, trauma, fever, infection, intercurrent illness, excessive exertion, alcohol ingestion, dehydration, fasting, menstrual periods. These episodes resolve spontaneously.
2. No significant enlargement of the liver.
3. Dyspepsia: nausea, vomiting and pain or discomfort in the abdomen, for which no cause is found.
4. Asthenia, general fatigue.
5. Splenomegalia is present in one variant of Gilbert's syndrome.

Laboratory findings

1. CBC: mild anemia; there are 2 types of Gilbert syndrome (with hemolysis or without).
2. Lactate dehydrogenase: levels are elevated in persons with hemolysis but are normal in patients with Gilbert syndrome
3. Liver function tests:
   - Unconjugated hyperbilirubinemia.
   - AST and ALT in the majority of cases are normal, rarely may be slightly increased
   - A familial increase in serum alkaline phosphatase levels (rare).
4. Phenobarbital test: serum bilirubin levels diminish when the enzyme activity is enhanced following phenobarbital administration.
5. Test with fasting (non-specific): a 2- to 3-fold rise in the plasma unconjugated bilirubin level within 48 hours of a fast that returns to normal levels within 24 hours of resuming a normal diet.
6. Test with radioactive-labeled chromium measures RBC survival because 60% of patients with Gilbert syndrome have a mild hemolysis.
7. Thin-layer chromatography: an accurate method to assess bilirubin fractions. In patients with Gilbert syndrome it shows high unconjugated bilirubin and an increased ratio of bilirubin monoglucuronide to diglucuronide, reflecting reduced UGT activity.
8. Polymerase chain reaction is a novel and rapid method of identifying genetic polymorphisms in the TATA box of the UGT1*1 gene using fluorescence resonance energy transfer.

Imaging

1. Abdominal ultrasonography for differential diagnosis.
2. Liver biopsy is rarely performed: the liver is normal; an increase in hepatocytes lipofuscin and an increase the smooth endoplasmic reticulum occasionally may be seen.
3. The bile investigation shows a modest increase in mono-conjugates of bilirubin that reflects reduced UGT activity.

In general, the diagnosis of this benign but not uncommon disorder is made by exclusion. The syndrome is suspected in a patient with low-grade unconjugated hyperbilirubinemia with the following:

1. No systemic symptoms
2. No overt or clinically recognizable hemolysis
3. Normal tests of routine liver function
4. Normal liver biopsy on light microscopy

The most commonly used diagnostic criteria of Gilbert syndrome are the following: (1) unconjugated hyperbilirubinemia noted on several occasions; (2) normal results from CBC count, reticulocyte count, and blood smear; (3) normal liver function test results; and (4) an absence of other disease processes.

Management: no specific therapy is needed; avoidance of precipitating factors is recommended. Prognosis is excellent in the majority of cases.

Crigler-Najjar Syndrome

Type I is the clinically severe form associated with neonatal jaundice and neurologic manifestations (originally described by Crigler and Najjar) and is due to absence of glucuronyl transferase activity as a result of a mutation in one of the common three regions of the *UGT1* gene (or lack of function of one or more isoenzymes of the transferase). Very high levels of unconjugated hyperbilirubinemia are observed at birth.

Type II (also called Arias syndrome) has more moderate clinical manifestations due to partial deficiency of glucuronyl transferase resulting from a mutation in the variable regions of the *UGT1* gene, resulting in lower levels of serum bilirubin and responsiveness to treatment with phenobarbital that induce the expression of UGT and decrease the serum bilirubin level of about 25%.

Both types are rare disorders with an autosomal recessive pattern of inheritance. Only a few hundred cases have been described in the world literature, and the real prevalence is unknown. They are thought to affect all races and both sexes equally.

Type I Crigler-Najjar syndrome

a. Infants develop high unconjugated bilirubin levels in the serum (340-855 µmol/L / 19.9-50 mg/dL). Conjugated bilirubin is absent in serum.

Affected infants usually die within the first year of life, although some patients have survived to the second or third decade of life. Death is usually from kernicterus (bilirubin encephalopathy). Clinical manifestations of kernicterus are hypotonia, deafness, oculomotor palsy, lethargy, and, ultimately, death.

b. Routine liver function tests are normal.

c. Urine analysis: bilirubin is not present.

d. Bile analysis (bile is collected through duodenal aspiration): because of the lack of UGT activity, no conjugated bilirubin is formed by the liver, hence no bilirubin is secreted into the bile and the bile is colorless (light yellow).

e. Oral cholecystography: the gallbladder is visualized because excretion of nonglucuronidated organic anions is normal

f. Liver histology is normal.

Patients with type II syndrome have only partial deficiencies of UGT and a less severe disorder. Jaundice may not appear until adolescence. Neurologic complications are uncommon, but can be induced by stress factors such as infection, anesthesia, or drug use.

a. Serum unconjugated bilirubin levels are lower (42.5-340 µmol/L / 6-48 mg/dL). Under stress conditions bilirubin levels may reach 680 µmol/L (40 mg/dL).

b. Routine liver function tests are normal.

c. Chromatographic analysis of bile is used to differentiate type I and type II syndromes: in type II, bile contains conjugated bilirubin, although the proportion of bilirubin monoglucuronide in bile is increased.

Management

- Phototherapy may temporarily, and transiently, reduce the unconjugated bilirubin level. It results in the formation of bilirubin photoisomers that can be excreted in bile without conjugation. The effectiveness of phototherapy decreases after age 3-4 years because the ratio of skin surface area to body mass is reduced. Oral calcium phosphate is recommended as adjuvant to phototherapy.
- Plasma exchange transfusion is indicated for emergent management of bilirubin encephalopathy. It removes the bilirubin-saturated albumin and provides free protein, which draws bilirubin from the tissues.
- Plasmapheresis is used during crises to achieve rapid bilirubin clearance.
- Inhibitors of heme oxygenase, such as tin protoporphyrin or tin-mesoporphyrin, may be helpful in reducing bilirubin levels emergently, but the effect is short-lived.
- Phenobarbital has no effect in type I disease, since the enzyme defect is complete and no drug induction is, therefore, possible. It may be used for the treatment of patients with type II syndrome and administered at doses of 3-6 mg/kg/d orally or intravenously. A response should be expected within 2-3 weeks. A similar benefit can be observed with clofibrate (500 mg PO qid), which is associated with fewer adverse effects.
- Orthotopic liver transplantation

Dubin-Johnson Syndrome (DJS)

This relatively rare disorder is called chronic idiopathic jaundice. It was first described independently in 1954 by Dubin and Johnson and by Sprinz and Nelson. It is benign autosomal recessive disorder caused by a mutation in the gene responsible for the human canalicular multispecific organic anion transporter (cMOAT) protein. This protein mediates ATP-dependent transport of organic anions across the canalicular membrane of the hepatocyte. A defect in the cMOAT protein results in impaired hepatobiliary transport of non-bile salt organic anions and is thought to be responsible for the conjugated hyperbilirubinemia and for the accumulation of hepatocellular pigment (dark pigment in the centri-lobular region of the liver cells).

Incidence: DJS occurs in all races, but prevalence is highest among Iranian Jews (1:300). This group may have an associated deficiency in clotting factor VII that is not observed in other populations.

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Patients with Dubin-Johnson syndrome may be asymptomatic or have vague constitutional or gastrointestinal symptoms.

Signs and symptoms

1. Non-pruritic jaundice (the degree of jaundice is increased by intercurrent illness, oral contraceptives and pregnancy)
2. Often, the liver is slightly enlarged, sometimes spleen is enlarged
3. Mild hepatic tenderness

Laboratory findings

1. Conjugated hyperbilirubinemia, with total bilirubin levels 51 to 257 µmol/L (2.9 to 15 mg/dL). However, with the more accurate method (high-pressure liquid chromatography) these patients have been shown also to have significant levels of serum unconjugated bilirubin
2. Urinary coproporphyrin levels: DJS patients excrete urine with predominance of coproporphyrin I and have increased ratio of coproporphyrin I to coproporphyrin III (CPI/CPIII ratio). Coproporphyrins are byproducts of heme biosynthesis. Normally, coproporphyrin I is preferentially excreted in bile, whereas coproporphyrin III is preferentially excreted in urine. Total urinary coproporphyrin levels can be increased in patients with different hepatobiliary disorders, but only patients with DJS have an increased CPI/CPIII ratio in the urine, therefore this finding is considered to be a pathognomonic for DJS.
3. Serum alkaline phosphatase, aminotransferases, albumin levels are within normal ranges.
4. Complete blood count and reticulocyte count usually are normal.
5. Prothrombin time is usually normal, but it can be prolonged if factor VII deficiency is present.
6. Oral and intravenous cholangiography fails to visualize the biliary tract.
7. Hepatobiliary scans: intense and prolonged (up to 120 minutes after IV dye injection) visualization of the liver with delayed (up to 90 minutes, normally within 30 minutes after dye injection) or no visualization of the gallbladder. This combination is also pathognomonic feature of DJS.
8. Late rise in the plasma sodium sulfobromophthalein elimination curve at 90 min. This is caused by the reflux of bilirubin from the liver.
9. Laparoscopy: liver is black.
10. Liver biopsy: a granulated brown or black pigment, most pronounced in the centrilobular zones (melanin-like). Certain diseases (e.g., viral hepatitis) can cause the pigment to disappear. The pigment reaccumulates slowly once the acute process is resolved.

Treatment: DJS is a benign disorder and does not require any specific therapy. Use of phenobarbital is not recommended.

Rotor syndrome

Rotor syndrome is another disease with conjugated hyperbilirubinemia caused by abnormal bilirubin uptake and defected bilirubin handling in the liver. Clinical manifestations, laboratory findings and prognosis are similar to those in DJS, but, in contrast to DJS, the syndrome has probably autosomal dominant pattern of inheritance and there is not melanin-like pigment deposition in the livers, which helps to differentiate these two diseases.

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ВРОДЖЕНІ ГІПЕРБІЛІРУБІНЕМІЇ: ДІАГНОСТИЧНИЙ ПІДХІД ТА МЕНЕДЖМЕНТ. ЛЕКЦІЯ ДЛЯ АСПІРАНТІВ, СТУДЕНТІВ-МЕДИКІВ

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Резюме. Гіпербілірубінемія проявляється жовтяницею, коли рівень загального білірубіну сироватки перевищує 34 ммоль/л. Існує декілька типів переважно коньгованої та переважно неконюгованої гупербілірубінемій. За пропонований діагностичний підхід до пацієнта з жовтяницею чи гіпербілірубінемією. Для синдрому Жільбера, синдрому Кріглера-Наджара, синдрому Дабіна-Джонсона та синдрому Ротора описані клінічні прояви, діагностичні критерії та принципи лікування.

Висновок. Вроджені гіпербілірубінемії є рідкісними синдромами, але диференціюються від інших уражень печінки з труднощами.

Ключові слова: вроджені гіпербілірубінемії, синдром Жільбера, синдром Кріглера-Наджара, синдром Дабіна-Джонсона, синдром Ротора.