Biochemistry and Pathophysiology of the liver. Jaundice.

Methodic materials for international students (IV-VI year)

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Published: 2004

Metabolism of bilirubin

Bilirubin is the major bile pigment in man and is formed as an end-product of the catabolism of haem-containing proteins.

Bilirubin is a tetrapyrrole compound formed when the ring structure of haem is broken open by microsomal *haem oxygenase*, thus liberating a bridge carbon as carbon monoxide (CO) (because of that total production of CO is used to estimate the rate of bilirubin formation in healthy persons).

Production: the major source of bilirubin in man is from **breakdown of red cells.**

Transport in plasma: Bilirubin is transported from its site of production to the liver **bound to albumin**.

Why? The molecular configuration of bilirubin secludes potentially polar groups and renders it non-polar and lipophilic. Even physiological concentrations of plasma bilirubin vastly exceed its solubility in water (4-7 nmol at pH 7.4) and are only achieved because of protein binding.

How? Each albumin molecule has one binding site for bilirubin which is of such high affinity and two additional binding sites of moderately high affinity and a third group of low-affinity binding sites.

However, when bilirubin : albumin ratios exceed 1 : 1, binding affinities are such that there is a disproportionate increase of the unbound freely diffusable fraction of bilirubin in plasma.

What substances are influencing bilirubin-albumin interaction? the agents that compete with bilirubin and displace it from the binding sites on albumin (e.g. salicylates, sulphonamides, diazepam, vitamin K analogues, and contrast media for cholangiography).

Albumin and conjugated bilirubin: In cholestatic jaundice some conjugated bilirubin becomes covalently bound to serum albumin. Thus, in spite of the resolution of obstructive jaundice is initially rapid due to clearance of dissociable bilirubin it is followed by a much slower phase corresponding to disappearance of the irreversibly bound albumin-bilirubin complex.

Bilirubin in liver:

Uptake of bilirubin by the liver is rapid and occurs independently of albumin uptake though involving interaction of the hepatocyte membrane with the albumin : bilirubin complex.

Regulation of liver uptake: Up-regulation by oestrogens and down-regulation by testosterone of the high-affinity transporter protein that mediates bilirubin uptake via the sinusoidal membrane. This may account for females generally having a lower serum bilirubin and for the tendency for Gilbert's syndrome to present in postpubertal males.

Within the hepatic cell, bilirubin is bound by ligandin (glutathione S-transferase B) and Z protein (also known as fatty acid-binding protein).

Competing agents: other organic anions, including bromsulphthalein, indocyanine green and cholecystographic agent, *compete with bilirubin for hepatocyte uptake and for binding to ligandin and Z protein*.

Regulation of these competing agents clearance: enhanced by phenobarbital, which increases ligandin synthesis, and reduced by fasting or oestrogen therapy, which diminish hepatic levels of ligandin and Z protein.

Conjugation:
Within the liver cell, free bilirubin is rendered water soluble by conjugation with *glucuronic acid.* This reaction is catalysed by *the microsomal enzyme glucuronyl transferase or UDPGT* (bilirubin uridine diphosphate glucuronate glucuronyl transferase).

The major conjugate in human bile is bilirubin diglucuronide, with lesser quantities of *monoglucuronide* and other conjugates, for example *glucosides and xylosides*.
Ratio of mono- and diglucuronide proportion in bile:

- monoglucuronide formation is favoured by a high bilirubin load, low enzyme activity or microsomal injury.
- diglucuronide formation is increased by enzyme induction by phenobarbitone

Biliary excretion
The rate-limiting step is canaclicular excretion.
Transport of bile acids and bilirubin into the canaliculus appear to be mediated by separate transporter proteins.
Thus, in the Dubin-Johnson syndrome, transport kinetics for bile acids are usually normal though there is a marked excretory defect for the organic anions bilirubin, bromsulphthalein, and indocyanine green.

Urobilinogen metabolism
Metabolism of bilirubin by intestinal bacteria produces a number of breakdown products, which include the readily absorbable, *water-soluble urobilinogen*.
Most of the absorbed urobilinogen is immediately extracted by the liver and excreted in bile, thus undergoing an enterohepatic circulation.
The small fraction of urobilinogen that passes through the liver to enter the systemic circulation is available for urinary excretion.
An increased urinary urobilinogen output may be caused by a diminished hepatic fractional extraction rate, when its detection is a very sensitive index of early liver disease.
Alternatively, increased urinary urobilinogen may occur in the face of a normal hepatic fractional extraction rate when it has been produced and absorbed in proportion to excessive bilirubin production, e.g. in *haemolysis.* If there is complete biliary obstruction, absence of bile from the gut will be reflected by absence of urobilinogen from the urine.

Clinical syndromes related to liver diseases

Jaundice is the yellow discoloration of sclerae, mucous membranes, and skin caused by *accumulation of bilirubin due to its* either excessive production or defective elimination. Involvement of the sclerae helps distinguish jaundice from other causes of pigmentation such as melanosis, hypercarotinaemia, and mepacrine therapy.

Types of Jaundice

1. haemolytic (lemon-yellow)
2. hepatocellular (orange-yellow)
3. cholestatic (greenish-yellow)

Causes of jaundice

Unconjugated			**Conjugated**
excessive bilirubin pro-	**reduced hepatic up-**	**reduced hepatic conjuga-**	**reduced excretion of conju-**

duction	take of bilirubin	tion	gated bilirubin
haemolytic anaemias	Gilbert's syndrome	neonatal jaundice	viral hepatitis
Marked degree of ineffective erythropoiesis	severe congestive heart failure	Crigler-Najjar syndrome (specific deficiency of glucuronyl transferase)	Drug-related jaundice
intramedullary destruction of red-cell precursors (for example, in thalassaemia and pernicious anaemia)	portocaval shunts	Inhibition of the activity of glucuronyl transferase by steroids excreted in maternal milk - rare cause of neonatal jaundice	Inflammatory, granulomatous or neoplastic infiltration of the liver
	Some drugs (rifampicin etc)		congenital defects of hepatic excretion in the Dubin-Johnson and Rotor syndromes.
			extrahepatic bile duct obstruction by gallstones, pancreatic carcinoma, and other disorders

2. Hepatocellular damage (cytolysis syndrome):

Clinical:

- Jaundice

Laboratory:

rise in the activity of serum aspartate aminotransferase (AST), and alanine aminotransferase (ALT) and lactate dehydrogenase (LDH, especially LDH5-isoenzyme). However, these tests are not fully specific (AST and LDH can reflect muscles (including cardiomyocytes) and hepatic damage). *Cytolysis syndrome also include the rise of serum ferritin and iron.*

3. Cholestasis of both intra- and extrahepatic origin*:*

Cholestasis - a constellation of clinical, biochemical, and histological findings in which the predominant abnormality is a failure of bile secretion.

Causes:

- obstruction of the extrahepatic biliary system
- obstruction of intrahepatic interlobular bile ducts (e.g. with primary biliary cirrhosis) and sclerosing cholangitis or parasitic infestations.
- intrahepatic cholestasis due to secretory failure at the level of the hepatocellular canaliculus in the absence of any obstruction to flow within the bile ductules or ducts. This is seen with drug-induced cholestasis and occasionally when liver disease is due to alcohol or viral hepatitis.

Morphology

1. recent and complete biliary obstruction:

- oedematous portal tracts
- bilirubin in hepatocytes
- plugs of inspissated bile within canaliculi, especially in centrolobular zones.

2. Prolonged cholestasis with partial obstruction (as in early primary biliary cirrhosis or primary sclerosing cholangitis):

- accumulation of copper-associated protein within periportal hepatocytes
- bile-ductular proliferation at the periphery of portal tracts
- and portal fibrosis.

Clinical manifestations:

- itching (when biliary obstruction is incomplete, pruritus is often first to appear and may be the sole feature for an extended period of time)
- jaundice
- dark urine (due to the increase of bilirubin and urobilin levels in urine)

- pale stools
- absence or diminution of intestinal bile acids may lead to steatorrhoea, and if cholestasis is prolonged, deficiency states affecting the fat-soluble vitamins A, D, E, and K may be anticipated.

Laboratory and instrumental findings:

1. The earliest detectable abnormality may be elevation of serum bile-acid levels
2. Increase of bilirubin level, 80% of which due to the increase of conjugated fraction
3. *Serum alkaline phosphatase (SAP).* Electrophoresis permits discrimination between isoenzymes of alkaline phosphatase that are characteristic of liver, bone, kidney, placenta, and intestinal mucosa.
4. *Serum 5-nucleotidase* is more specific to liver than alkaline phosphatase and its estimation or that of the more commonly available
5. *Gamma-glutamyltransferase (transpeptidase)* can be substituted for isoenzyme analysis in order to discriminate between hepatic and bony origins of raised SAP activity. Measure of the serum bile-acid concentration has a high specificity for detection of minor degrees of hepatic dysfunction. The sensitivity is enhanced by performing estimations on blood taken 2hrs after a fatty meal, when hepatic clearance has been stressed by the bile-acid load returning in the portal blood as part of the enterohepatic circulation.
6. Rise of serum level of *Leicinaminopeptidase*
7. *Cholesterole metabolism changes (cholesterol, phospholipids and beta-lipoproteins rise in blood; in marked cholestasis the lipoprotein X appears – lipoproteid complexes with fragments of plasma membrane).*
8. *In urine bilirubin and urobiline level are increased.*

4. Minor hepatic failure syndrome

<table>
<tr><th>Clinical manifestations</th><th>Laboratory test</th></tr>
<tr><td colspan="2">Decrease of detoxication function of liver</td></tr>
<tr><td rowspan="2">Neural system affection:
- hepatic laziness (the patient spends most of the day in bed)
- sleepiness
- hypohondric condition
- weakness
- trend to syncopes</td><td>galactose elimination capacity (measuring hepatic cytosolic functional mass):
rate of fall in its blood concentration following a single bolus intravenous injection of galactose (0.5 g/kg); blood samples taken at 5-min intervals between 20 and 40min later.</td></tr>
<tr><td>Bromsulphthalein and indocyanine green (compete with bilirubin for hepatocyte uptake). Bromsulphthalein can rarely cause anaphylactoid reactions. Indocyanine green is not metabolized by the liver but its clearance can be used to measure hepatic blood flow because the liver is solely responsible for its removal from blood.</td></tr>
<tr><td rowspan="2">Disturbed inactivation of estrogens, serotonin and other substances:
- palmar erythema
- vascular “stars” on the skin of nose, fossa jugularis, shoulders, neck, upper chest and back (enlargement of arterious-venous anastomoses)
- gynecomastia and absence of hair on chest in men</td><td>^{1,4}C-aminopyrine breath test is done by giving the patient a small amount of radioactive aminopyrine by mouth and measuring radioactive $^{1,4}CO2$ released by N-demethylation in trapped breath 30 and 60min later.</td></tr>
<tr><td>Like antipyrine and caffeine clearance and the MEG-X (monoethylglycine xylidide) test, which measures N-de-ethylation of lignocaine, the result is a measure of hepatocyte P450 enzyme activity.</td></tr>
<tr><td colspan="2">Decrease of synthetic function of liver</td></tr>
<tr><td>Gums bleeding and petechia appearance</td><td>Decrease of levels of clotting factors, synthesized by the liver (II, V, VII, IX, and X)</td></tr>
<tr><td>-</td><td>Decrease of circulatory proteins and substances that are exclusively synthesized by the liver: albumin, alpha-lipoproteins, cho-</td></tr>
</table>

	lesterol level, cholinestheraze activity

3. **Dyspepsia:**

- loss of appetite, nausea, more rare – vomiting, diarrhea (a certain role in development of this syndrome plays intestinal disbiosis)

4. **Intoxication and asthenic syndrome**

Weakness, increase of temperature

5. **Liver enlargement:** usually present in most of the diseases; the characteristics of liver depends on the type of the disease (soft liver margin in case of steatosis, hepatitis; dense – in case of cyrrhosis etc)
6. **Spleen enlargement:** in case of either substances deposition in spleen (Dubin-Johnson syndrome) or portal hypertension
7. **Mesenchimal-inflammatory syndrome:** in case of inflammatory liver diseases – fever, increase of ESR, C-reactive protein, increase of seromucoid level, gamma-globulins, serum immunoglobulins

Unconjugated hyperbilirubinaemia

Gilbert's syndrome

In 1901, Gilbert noticed a familial occurrence of hyperbilirubinaemia in the absence of apparent liver disease.

Epidemiology and genetics:

1-5% of the population; autosomal dominant type; appears in young age. Men:women ratio is 8-10:1, the main cause of men dominance is up-regulation of hepatic uptake of bilirubin by oestrogens and down-regulation by testosterone.

Biochemistry, pathogenesis and morphology:

The hepatic uptake and hepatic glucuroniltransferase activity are usually diminished, suggesting a primary defect in glucuronidation in microsome fraction of hepatocytes.

Morphology: Liver histology is grossly normal, though an increase of yellowish-brown pigment lipofuscin may be seen especially in centrolobular hepatocytes. Electron microscopy suggests heterogeneity between patients, with evidence of mitochondrial changes and proliferation of the smooth endoplasmic reticulum in some. Signs of liver steatosis may be also seen.

Symptoms and signs:

- intermittent jaundice (mostly sclerae, more rare - skin), which appears after stress, physical exercise, some drugs intake, alcohol intake, viral infection, concomitant diseases etc; in 10-20% of patients – the only symptom of the disease
- in exacerbation may be non-specific complaints:
- abdominal pain
- weakness, malaise
- mild dyspepsia (absence of appetite, nausea, stools disorders)
- in some patients mild/moderate increase of liver size may be present
- biliar disorders may be present (dyskinesia, chronic cholecystitis)
- spleen size is normal

Laboratory signs:

- free (unconjugated) bilirubin level in serum is increased in absence of haemolysis signs; plasma bilirubin levels fluctuate markedly, but are usually not sufficiently high to produce jaundice
- increase of LDH5 may be present in serum
- mild decrease of bromsulphalein clearance may be present (defect in hepatic uptake of organic anions); in some patients clearance of indocyanine green is defective, though this organic anion is excreted unconjugated
- mild/moderate haemoglobin level increase may be and mild decrease of ESR

- provocation test: 50 mg of nicotinic acid may be injected intravenously. Unconjugated serum bilirubin is increased by nicotinic acid as the result of complex mechanisms that include increased erythrocyte fragility, increased splenic haem-oxygenase activity, and increased formation of bilirubin in the spleen. An increase of unconjugated serum bilirubin of more than 17mgr/mol/l is highly suggestive of Gilbert's syndrome and the concentration versus time curve shows a significant delay in the clearance of unconjugated bilirubin at the end of the test compared to controls. This provocation test is especially recommended in patients with suspected Gilbert's syndrome whose serum bilirubin is normal at the time of investigation.
- liver biopsy is required when there is reasonable doubt about the diagnosis, but is not essential.

Natural course of the disease and prognosis:

- Rapid dynamics of patients' condition during the exacerbations: disappearance of complaints, normalizing of liver size in several days or weeks
- Remission duration may be different, up to several years
- The prognosis is excellent, and there is no substantive evidence for the association of Gilbert's syndrome with other more serious disorders. Patients require no more than the reassurance derived from an understanding of the commonness of their condition and its totally benign nature.

Crigler-Najjar syndrome

The Crigler-Najjar syndrome results in severe unconjugated hyperbilirubinaemia in the absence of any other evidence of hepatic dysfunction or haemolysis. Where they have been studied, hepatic clearance of bromsulphthalein and indocyanine green, red-cell survival, and early labelled bilirubin peak are all normal.

The underlying defect appears to be a deficiency of UDPGT activity. There are two genetically distinct groups, types I and II.

TYPE I

In type I Crigler-Najjar there appears to be a *complete absence of UDPGT activity*, and no bilirubin glucuronide is formed though other compounds may be glucuronidated by the liver. Severe unconjugated hyperbilirubinaemia occurs in the **neonatal period,** leading to kernicterus (affection of brain by high bilirubin level) and early death in most infants. A minority survives the neonatal period without apparent brain damage but may also succumb in later childhood.

- Genetics: autosomal recessive mode of inheritance is suggested. Heterozygotes are usually not jaundiced.

The diagnosis is based on clinical and laboratory findings.

- no Rhesus, ABO or other blood-group incompatibility and no haemolysis.
- liver function tests are normal
- no bilirubin in the urine
- bilirubin conjugates are not present in bile, which may be colourless.
- colour of faeces is normal
- urobilin is present though quantitatively diminished in the faeces. The explanation for this is unclear, but is thought to result from diffusion of a small amount of unconjugated bilirubin into the bowel lumen across the intestinal mucosa and biliary tract.

TYPE II

Type II Crigler-Najjar is associated with only *partial deficiency of UDPG activity*, and is a less well-defined entity than type I embracing those unconjugated hyperbilirubinaemias of hepatic origin that are intermediate between Gilbert's syndrome and type I Crigler-Najjar.

Inheritance:

- Familial occurrence is common.
- Gilbert's syndrome is frequently found in relatives

Clinical manifestations

- Jaundice is usually of less acute onset and less severe than seen in type I, and *occasionally jaundice may not be apparent in early childhood*.
- Kernicterus is rare.
- Bile contains bilirubin and therefore is deeply pigmented.

TREATMENT

- In Crigler-Najjar type II syndrome the time course of the response to **phenobarbital** therapy suggests than an enzyme with a relatively long half-life is being induced.
- The lack of any such responsiveness to phenobarbital in type I, is a rare indication for replacing a histologically normal liver by transplantation
- In type II, phenobarbital treatment reduces plasma bilirubin levels, which patients may welcome for its cosmetic effect.
- **Phototherapy** is effective in alleviating hyperbilirubinaemia in infants. Bilirubin has an intense absorption band in the visible spectrum between 425 and 475nm, and exposure of the skin to light of this wavelength reduces the plasma bilirubin concentration. A series of highly polar, water-soluble derivatives of bilirubin result from its photodegradation, and these pass readily into urine, bile or across the intestinal wall. This treatment has become established as a means of preventing kernicterus in neonatal units. However, it is probably impractical as a lifelong measure, even in patients with Crigler-Najjar type I syndrome, and its freedom from undesirable side-effects in the long term is unproven. Photodegradation may be enhanced by riboflavin.

Physiological jaundice of the newborn

A rise in plasma bilirubin concentration occurs very commonly in the first few days of life, but infants born prematurely with low birth weights are most likely to become jaundiced.

In utero, bilirubin is transported across the placenta and removed by the maternal liver.

After birth:

- the fetal liver is immature because of low levels of ligandin, glucuronyl transferase activity, and capacity for excretion of conjugated bilirubin into bile
- glucuronyl transferase activity increases exponentially to reach adult levels by 14 weeks of age, regardless of full-term or premature birth.
- So, during the initial period of life the liver it may be incapable of maintaining the increased after birth plasma bilirubin levels
- Susceptibility to hyperbilirubinaemia in the neonate may be compounded by a shortened red-cell lifespan, reduced caloric intake or shunting past the liver through a patent ductus venosus.

Thus, plasma bilirubin levels must be monitored in jaundiced infants because of the risk of kernicterus. Though levels in excess of 340mgr/mol/l have, traditionally, been thought to constitute a risk of kernicterus, recent work suggests that plasma levels half as high may be associated with some impairment of subsequent psychomotor development.

This problem can be averted by reduction of plasma bilirubin concentrations with phototherapy or exchange transfusions.

In addition, reabsorption of unconjugated bilirubin, formed in the intestine by the action of beta-glucuronidase, may be prevented by the oral administration of a non-absorbable agar, which binds the bilirubin. Drugs that displace bilirubin from albumin should be avoided (salicylates, sulphonamides, diazepam, vitamin K analogues, and contrast media for cholangiography).

Transient familial neonatal hyperbilirubinaemia

Occasionally there is a familial tendency to develop neonatal hyperbilirubinaemia, and affected infants are at risk of developing kernicterus even following full-term gestation.

There is no haemolysis and the onset of jaundice occurs earlier than is seen with breast-milk jaundice. The management is the same as for physiological jaundice. Jaundice is transient and the long-term prognosis is excellent.

Breast-milk jaundice
A small number of infants have been reported in whom jaundice due to unconjugated hyperbilirubinaemia has occurred, apparently as a result of breast feeding. Kernicterus is not a complication.
There appears to be an icterogenic factor in breast milk that is not detectable in maternal serum (possibly - steroid pregnanediol, which is an inhibitor of glucuronyl transferase)
Siblings of affected individuals are also liable to jaundice if breast fed.

Conjugated hyperbilirubinaemia
Conjugated hyperbilirubinaemia results from reflux into plasma of bilirubin which has previously been taken up and conjugated by the liver. In most instances it is found in association with retention of other biliary constituents such as bile acids, but typical families have been described in which defective excretion of conjugated bilirubin occurs as an isolated defect.
The Dubin-Johnson syndrome
This syndrome is a rare type of pigment hepatosis with autosomal dominant type of inheritance, mostly occurring in males and caused by the disturbances of bilirubin excretion from hepatocyte and thus bilirubin regurgitation.
Morphology:
- the liver is deeply pigmented, and the diagnosis may be suspected if a liver biopsy specimen appears black to the naked eye (liver may be either greenish-grey or brounish-black).
- liver cells contain much granular pigment that is not bilirubin and has been variously ascribed to lipofuscin and melanin (liver melanosis due to adrenalin metabolism disturbances); melanin deposits are localized peribiliary
- histological structure of liver is normal
- pigment deposition is also revealed in spleen

Clinical manifestations:
- Jaundice: usually constant, varying in intensity with increase in exacerbations
- Itching
- Anorexia, malaise, easy fatiguability
- Dyspepsia: nausea, occasional diarrhea
- Local signs: right hypochondrial pain, moderate increase of the liver size
- Splenomegaly may be present
- Bile regurgitation is a factor predisposing to bile stones formation; in this case bile colic and other symptoms of bile stone disease will be present

Laboratory and instrumental findings:
- there is an increase of conjugated bilirubin in serum; the level varies widely and fluctuates in the individual patient
- Bilirubin and excess urobilinogen are found in the urine in the absence of any haemolysis or abnormalities of liver function tests (however, moderate/mild increase of ALT, AST and alkaline phosphatase may be present)
- non-visualization of the gallbladder with cholecystographic media (oral and i.v. cholecystography)
- Bromsulphthalein clearance: early plasma disappearance of bromsulphthalein appears normal and retention at 45 min may not be markedly deranged; however, plasma levels subsequently rise so that at 90 min (or 2 hours) they exceed those at 45 min, due to reflux of glutathione-conjugated bromsulphthalein.
- Urinary coproporphyrin excretion: in homozygous patients the ratio of urinary coproporphyrin I to coproporphyrin III is at least 4 : 1, compared to a ratio of approximately 1 : 3 in normal subjects. In heterozygous carriers there are The intermediate ratios. Similarly increased ratios occur in erythropoietic porphyria but the markedly increased overall excre-

tion of coproporphyrins seen in that situation are not a feature of the Dubin-Johnson syndrome.

Prognosis and treatment: There is no recognized treatment for the condition, which is benign, and the patient can be reassured accordingly. Very rarely there has been a coexistent dyserythropoietic anaemia or a defect in blood coagulability.

Rotor's syndrome

Rotor described the familial occurrence of conjugated hyperbilirubinaemia in natives of the Philippines. Originally it was considered to be a variant of the Dubin-Johnson syndrome, now thought to have a different pathology with demonstrable uptake and storage defects..

Inheritance: Rotor's syndrome is familial pigment hepatosis with autosomal-dominant type of inheritance.

Pathogenesis and morphology:

There is no apparent abnormality of bile acid transport and other liver function tests are normal. The bilirubin excretion defect is less marked, deposits of dark pigment are absent. Morphologically the signs of liver steatosis may be present.

Clinical manifestations

- chronic or intermittent jaundice appearing from childhood
- malaise, tiredness etc
- dyspepsia may be present (loss of appetite etc)
- right hypochondric pain may be present
- mild liver enlargement is usually present

Laboratory and instrumental findings

- increase of conjugated plasma bilirubin
- in exacerbation, level of AST, ALT and alkaline phosphatase may be increased
- increase of coproporphirines in urine (typical)
- decrease of bromsulphalein clearance without the late increase of its level in blood
- cholecystography is normal

Prognosis: benign

Gilbert syndrome, Dubin-Johnson syndrome and Rotor syndrome are included into the group of the diseases called Pigment hepatoses:

Pigment hepatoses are benign enzymopathies caused by hereditary disturbances of bilirubin metabolism and manifested by hyperbilirubinemia, intermittent or constant jaundice without marked changes of structure and function of the liver.

Differential diagnosis of pigment hepatoses

	Gilbert	**Dubin-Johnson**	**Rotor**
Age of onset (typical)	Teenagers; young	Teenager; young (90% - below 25)	Teenagers; young
Pain in right hypochondric region	Rare, aching	Often; colic pain may be present	Often; colic pain may be present
Itching	Absent	Rare, weak	Usually absent
Liver increase	Typical, mild	Typical, mild	Rare
Spleen increase	Absent	Possible	Rare
Serum bilirubin level	Mostly protein-bound (free)	Mostly conjugated	Mostly conjugated
Bilirubinuria	Absent	Present	Present
Coproporphirines	Absent	Maybe mild increase	Typical increase
Glucuroniltransferase activity	Decreased	Normal	Normal
Bromsulphalein test	Normal in most of cases; may be mild	Decrease of clearance with late (90 min-2hrs)	Decrease of clearance without the second

	decrease of clearance	second peak of increase of substance in blood	peak
Cholecystography	Normal	Absence or delay in contrast filling of gallbladder biliary tract	Oral cholecystography is normal
Liver biopsy	Normal or lipofuscin deposits; signs of steatosis	Dark pigment deposits	Normal
Prognosis	Benign	Benign	Benign

Intrahepatic cholestasis

Vanishing bile-duct syndrome

Severe progressive cholestasis may ensue in conditions that destroy bile ducts of predominantly interlobular size.

Morphology

Normally, portal tracts contain arterioles and bile ducts of approximately equal size and in a ratio of 1 : 1. In vanishing bile-duct syndrome: sensitive and specific stains for biliary epithelium reveal none in more than a half, and occasionally all, portal tracts.

Causes:

- the process is always insiduous in conditions such as primary biliary cirrhosis, primary sclerosing cholangitis, sarcoidosis, and mucoviscidosis
- can also occur either insidiously or abruptly in liver allograft rejection, graft-versus-host disease and extremely rarely in Hodgkin's disease and cholestatic drug reactions (e.g. reactions to flucloxacillin).

Prognosis and treatment: when the syndrome is severe, liver failure supervenes and liver transplantation is the only curative option.

Benign recurrent intrahepatic cholestasis

Characterized by recurrent attacks of cholestasis in the absence of any mechanical biliary obstruction and with restoration of completely normal hepatic structure and function between attacks.

Inheritance: benign recurrent intrahepatic cholestasis has a familial incidence but no hereditary pattern is apparent. One family has been reported in which there were several members with benign recurrent intrahepatic cholestasis, cholestasis of pregnancy or contraceptive steroid-induced cholestasis, which suggests a related mechanism in these conditions. Because of the early changes in bile-acid metabolism in each attack it has been postulated that the bile acids have a primary pathogenetic role.

Epidemiology:

- relatively rare condition
- affects males more than females
- the first attack usually occurs during childhood or adolescence:

Morphology:

Centrilobular cholestasis.

Electron microscopy: - changes common to many forms of cholestasis: canalicular dilation with loss of canalicular microvilli, widening of the pericanalicular ectoplasm, enlargement of the Golgi, and proliferation of the smooth endoplasmic reticulum.

Clinical manifestations:

Patients tend to recognize a precise pattern common to all of their attacks, though the features vary between individuals.

1. First symptoms of attack:
- vague, right hypochondrial pain
- malaise
2. Pruritus: usually precedes jaundice appearance
3. Jaundice: may be present but not obligatory

Laboratory and instrumental diagnosis:
- increased serum bile acids level
- increased alkaline phosphate levels a
- conjugated hyperbilirubinaemia
- bilirubin is present in the urine
- urinary urobilinogen is extremely low.
- steatorrhoea due to cholesthasis
- significant hypoprothrombinaemia with bruising due to cholestasis.
- **If the diagnosis is not established, cholangiography may be used to rule out mechanical obstruction.**

Prognosis and natural course of the disease:
- attacks last for several weeks or months and are separated by intervals of normality lasting many months or years
- benign, non-progressive nature of their disease.

Treatment:
- ursodeoxycholic acid is useful both for prophylaxis and in controlling attacks in some patients.
- despite their wide usage there is no convincing benefit from corticosteroids or cholestyramine.

Cholestasis of pregnancy: clinical manifestations resemble benign recurrent intrahepatic cholestasis
Incidence: usually in the third trimester of pregnancy.
Epidemiology and inheritance:
- relatively strong familial tendency
- the precise mode of inheritance is unclear.
- these women have two to three times the prevalence of gallstones found in their unaffected peers of similar parity.
- Particularly high incidences has been observed in certain countries, notably in Scandinavia and Chile.

Pathogenesis
- The concurrence of cholestasis in the third trimester of pregnancy when oestrogen levels are at their highest, with a predisposition of the same subjects to develop cholestasis when given contraceptive steroids points to a pathogenetic role for oestrogens.
- The importance of progestogens is less certain.

Clinical manifestations and laboratory tests:
- Pruritus is usually the first symptom
- Cholestasis may progress to jaundice with unconjugated hyperbilirubinaemia, bilirubinuria, and low urinary urobilinogen levels.
- Hypochondrial pain is uncommon and fever is not a feature.
- Serum bile acids are raised from the onset of pruritus.

- Serum ALT is the most sensitive but becomes abnormal later and only in the more severely affected.
- Steatorrhoea may be present
- Hypoprothrombinaemia may occur, it can be corrected rapidly by parenteral administration of vitamin K.

Prognosis and natural course of the disease:
- Cholestasis resolves rapidly after parturition, normality is usually restored within 2 to 3 weeks.
- Incomplete resolution of the cholestasis after delivery may indicate underlying disease, such as primary biliary cirrhosis, that has been unmasked by pregnancy
- spontaneous labour occurs prematurely and, although outcome is usually benign, there is an increased risk of fetal distress and unexplained stillbirth.
- no long-term ill effects on infant or mother have been described
- There is strong tendency for cholestasis to recur in subsequent pregnancies , and these women are also predisposed to develop cholestasis if given oral contraceptive steroids.

Differential diagnosis:
- cholelithiasis, which has increased incidence during pregnancy
- viral hepatitis is a more common cause of jaundice during pregnancy. It is more common but with more marked cytolysis and less - cholestasis

Treatment:
- Reports of benefit from treatment with **S-adenosyl methionine** lack confirmation.
- **Ursodeoxycholic acid** reverses liver dysfunction and pruritus in some patients, but its protective effect on the fetus remains to be determined.
- **Cholestyramine,** even in high doses, is less beneficial.
- **Early delivery of the fetus following confirmation of lung maturity is recommended, at least in those with a prior history of fetal distress or stillbirth.**

Contraceptive steroid-induced cholestasis
Pathogenesis: see above
Clinical manifestations:
- Pruritus progressing to jaundice occurs in some women taking oral contraceptive steroids
- Symptoms usually appear during the first three monthly cycles, and recede spontaneously when the pill is discontinued.

Relation to other diseases with cholestasis:
- those who have experienced cholestasis on the contraceptive pill have a high likelihood of developing cholestasis of pregnancy and vice versa.
- If possible, oral contraceptive steroids should be avoided in women with a prior history of cholestasis of pregnancy or benign recurrent intrahepatic cholestasis.
- Rarely, progressive biliary disorders such as primary biliary cirrhosis or sclerosing cholangitis may first become symptomatic due to the unmasking effect of contraceptive steroids and also constitute contraindications to their use.
- There is no evidence to suggest an increased risk of oral contraceptive-induced cholestasis during convalescence from viral hepatitis.