REVIEW ARTICLE Egypt. J. Med. Hum. Genet. Vol. 9, No. 2, Nov. 2008

Cholestasis in neonates and infants

Amel Elfaramawy

Pediatris Department, Ain Shams University

ABSTRACT

The term cholestasis is Greek in origin, meaning bile stoppage. In its most overt form, cholestasis presents to the clinician as jaundice. However, jaundice is only the tip of the iceberg of cholestatic liver disease.
The incidence of neonatal cholestasis is estimated around 1 in 2500 live births. Cholestasis must always be considered in newborns with prolonged jaundice lasting more than 14-21 days. There are a number of contributing factors for neonatal cholestasis which include bile duct abnormalities; infections, such as sepsis and urinary tract infections; inherited and metabolic disorders; endocrine disorders; chromosomal disorders; toxicity from parenteral nutrition; vascular disorders; prematurity, lack of enteral feedings and medications. The most common causes of neonatal cholestasis are biliary atresia and idiopathic neonatal hepatitis.
The Aim : of this review is to discuss the pathophysiology, causes and approach of cholestasis.

Key Words:
Neonatal jaundice, neonatal hyperbilirubinemia, cholestatic liver disease, biliary atresia, metabolic causes of jaundice.

Corresponding Author:

Dr: Amel Elfaramawy
E-mail: amelhome4@gmail.com

DEFINITION

Neonatal cholestasis is defined as impaired bile formation or bile flow resulting in accumulation of biliary substances (bilirubin, bile acids and cholesterol) in blood and extrahepatic tissues. This can occur anywhere between the sinusoidal membrane of the hepatocyte and the ampulla of vater.

It is generally associated with a measured conjugated (direct-acting) bilirubin fraction of greater than 2 mg/dl or more than 20% of the total bilirubin.[1]

PATHOPHYSIOLOGY

The normal process of bile production involves two main processes: uptake of bile acids by hepatocytes from the blood and excretion of bile acids into the biliary canaliculus. Uptake of bile acids from sinusoidal blood is an active process at the sinusoidal membrane of the hepatocytes[2]. Na taurocholate co transporting polypeptide (NTCP) and organic anion transporting proteins (OATP) are the two main receptors involved in the uptake of conjugated bile acids by the liver cells. These receptors are also re-

sponsible for the transport of other anions like drugs and toxins through the hepatocellular membrane.

At the canalicular membrane, bile salt export pump (BSEP) and the multidrug resistant proteins MRP2 and MDR3 are involved in the secretion of bile acids into bile (Figure 1).

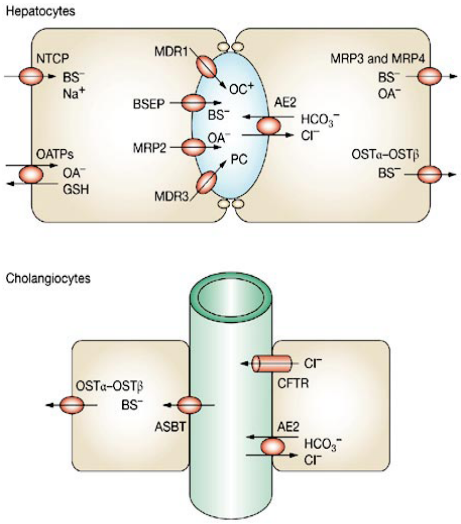

Fig. 1: Role of hepatic transporters in the formation of bile.[3]

Genetic defects in different transporters are recognized as the cause of a wide range of familial childhood cholestatic diseases, e.g. mutations in the gene for BSEP cause progressive familial intrahepatic cholestasis (PFIC) type 2. More surprisingly, is the recognition of the importance of mutations in transporters causing adult onset diseases such as intrahepatic cholestasis of pregnancy as it is associated in some cases with mutations in the gene for MDR3.[3]

Bile flow is low in the fetus and newborn as a result of a combination of immature bile acid synthetic and transport processes. The dynamics of the development process for human hepatocyte transporters have not yet been quantified, but plasma bile acids do not fall into the normal adult range until 6 months of age.[4]

CLINICAL PRESENTATION

This presentation is usually with prolonged jaundice. Newborn infants have pale stools and a history of persistently dark yellow urine confirms the presence of conjugated hyperbilirubinemia. The presence of pale stools is very sensitive for liver disease and even as an isolated finding should prompt immediate investigation. Acholic or white stools imply complete cholestasis with a consequently worse prognosis.

An alternative presentation is with bleeding due to vitamin K deficiency, as bruising, or with a devastating intracranial bleeding. Seizures may result from hypocalcaemia secondary to vitamin D deficiency or rarely due to hypoglycemia, the latter strongly suggests metabolic liver disease or hypopituitarism.[5]

Examination reveals hepatomegaly in most cases and splenomegaly in 50% of cases. Other helpful diagnostic clinical features may include stigmata of syndromic disorders, facial dysmorphic features, and evidence of congenital heart disease and manifestations of intra-uterine infection such as growth retardation or thrombocytopenia. Cutaneous cavernous haemangiomata may be associated with intrahepatic haemangiomata.[5]

Table 1 : Differential diagnosis of neonatal cholestasis.[6]

Classification	Example
I. Bile duct obstruction:	
- Extrahepatic disorders	- Biliary atresia, choledochal cysts, spontaneous perforation of common bile duct, Caroli's disease, bile duct stenosis, cholelithiasis, tumors/masses (intrinsic and extrinsic).
- Intrahepatic disorders	- Alagille's syndrome, nonsyndromic paucity of intrahepatic ducts, neonatal sclerosing cholangitis, congenital hepatic fibrosis, inspissated bile/mucous plug
II. Neonatal Hepatitis:	
- Viral	- Cytomegalovirus, herpes (simplex, zoster, human type 6), rubella, reovirus type 3, adenovirus, enteroviruses, parvovirus B19, hepatitis B, HIV
- Bacterial and parasitic.	Bacterial sepsis, UTI , listeriosis, tuberculosis, toxoplasmosis.
- Idiopathic neonatal hepatitis	
III. Cholestatic syndromes :	
- Progressive familial intrahepatic Cholestasis (PFIC)	caused by transport defect type 1 (Bylers disease, defect in FIC1, a P-type ATPase), type 2 (defect in BSEP, canalicular bile acid pump), type 3 (defect in MDR-3, a canalicular phospholipids transporter)
- Hereditary cholestasis with Lymphedema (Aagenaes syndrome)	
- Benign recurrent cholestasis (BRIC)	
- Dubin-Johnson syndrome	
- Rotor syndrome	
IV. Metabolic disorders:	
- ∞1-Antitrypsin deficiency	
- Cystic fibrosis	
- Neonatal iron storage disease	
- Amino acid disorders	- Tyrosinemia, hypermethionemia, mevalonate kinase deficiency
- Lipid disorders	- Niemann Pick disease, Gaucher's disease, Wolman's Disease.
-Urea cycle disorders	- Arginase deficiency
- Carbohydrate disorders	- Galactosemia, fructosemia, glycogen storage disease type IV.
-Mitochondrial disorders	
- Peroxisomal disorders	- Zellweger syndrome, infantile Refsum's disease, other enzymopathies
- Bile acid synthetic defects	- 3b-Hydroxy-D5-C27-steroid dehydrogenase isomerase, D4-3-oxosteroid 5b-reductase, oxysterol 7 ∞-hyroxylase
V. Endocrinopathies :	- Hypopituitarism (septooptic dysplasia), -Hypothyroidism.
VI. Toxic :	- Drugs, parenteral nutrition,
VII. Miscellaneous Associations:	Shock/hypoperfusion, histiocytosis X, neonatal lupus erythematosus, Indian childhood cirrhosis, autosomal trisomies, graft-versus-host disease, erythrophagocytic lymphohistiocytosis, extracorporeal membrane-oxygenation, venoocclusive disease.

EVALUATION OF CHOLESTASIS

Any infant presenting with jaundice beyond 2 weeks after birth should be immediately evaluated for cholestasis. A detailed history (including family history, pregnancy and delivery history and postnatal course) and physical examination could provide clues to a specific diagnosis.[7]

Once cholestasis is established, further investigations should be done in a stepwise manner to establish the specific cause of cholestasis (Figure 2). The investigations should first rule out conditions requiring immediate intervention like sepsis, metabolic disorders like galactosemia, glycogen storage disorders and other endocrinopathies. The next step is to look for biliary atresia. It is important to establish or rule out biliary atresia early because of better prognosis if the patient undergoes surgical intervention before 60 days of life. Then further investigations should be done to establish the cause of intrahepatic cholestasis (Table 1). The potentially extensive evaluation of an infant with cholestasis (Figure 2 and Table 1) should be individualized to efficiently and promptly establish a diagnosis.[8]

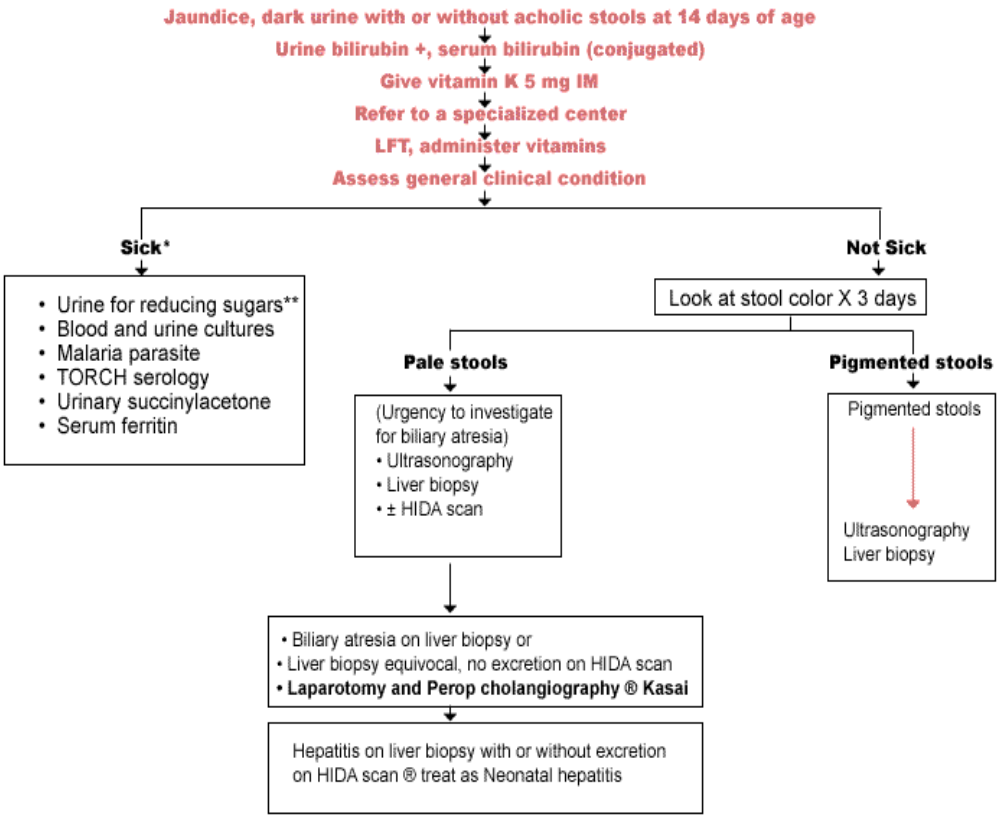

Fig. 2 : An approach to infant with Cholestasis.[8]

SPECIFIC DISEASES

Extrahepatic biliary atresia:
Biliary atresia is an idiopathic inflammatory process involving the bile ducts resulting in obstruction of the biliary tract, chronic cholestasis and progressive fibrosis and eventually leads to biliary cirrhosis. It accounts for approximately one-third of the cases of neonatal cholestasis and is the most common cause of liver transplantation in children.[9]

The incidence of biliary atresia has been estimated to be about 1:15,000. It is worldwide in distribution and occurs in all races, though more commonly in nonwhites. The etiology of biliary atresia is still unclear. Various studies have suggested a possible relationship between biliary atresia and viral infections like reovirus 3, rotavirus C and cytomegalovirus but this has not been conclusively proven. Increased risk of biliary atresia in family members of an affected individual has been noted and may suggest genetic etiology. Studies have shown that severe jaundice and death within 1 week of life occurs in the *inv* mouse, a transgenic mouse with deletion of the inversin gene and is associated with biliary atresia and complete abdominal situs inversus.[10]

There are two forms of biliary atresia:
1- Isolated biliary atresia, the more common form, also known as peri- or postnatal form.

2- biliary atresia associated with situs inversus and polysplenia syndrome, also known as the fetal or embryonic form. The polysplenia syndrome includes situs inversus, poly- or asplenia, cardiovascular malformations and anomalies of the portal vein and hepatic artery.[11]

Clinically, babies usually present with cholestasis 2-3 weeks after birth, and their birth weight is appropriate for gestation age, hepatomegaly is usually found on clinical examination. The stool is clay color and pruritus is rare before 5 months of age. Laboratory investigations usually show raised total and direct serum bilirubin, serum aminotransferases, alkaline phosphatase and gamma-glutamyl transpeptidase (GGT). Liver histopathology shows bile duct proliferation, bile lakes, cellular infiltrate, and fibrous tissue.[12]

T99c-labelled compounds of Iminodiacetic acid (e.g. HIDA) are simple ways of showing bile duct patency in suspected biliary atresia. Abdominal ultrasound occasionally shows triangular cord sign (TC sign), if the gallbladder is unidentified by ultrasound this may indicate obliteration of its lumen. Both percutaneous liver biopsy and percutaneous cholangiography support a diagnosis of biliary atresia. In most cases of biliary atresia, exploratory laparotomy is helpful in confirming the diagnosis.

Early biliary atresia requires Kasai portoenterostomy around 6 weeks of age. Post operative cholangitis is a well recognized complication of Kasai operation and usually it is treated with ampicillin and an aminoglycoside with or without clindamycin. Other complications include portal hypertension and fat soluble vitamin malabsorption which respond to fat soluble vitamin supplement. Liver transplantation can be life saving and should be done before the onset of irreversible hepatic decompensation.[12]

Another cause of extra hepatic cholestasis in infancy is choledochal cyst which is diagnosed by abdominal ultrasound and once the diagnosis is confirmed, surgical excision is the best therapy.

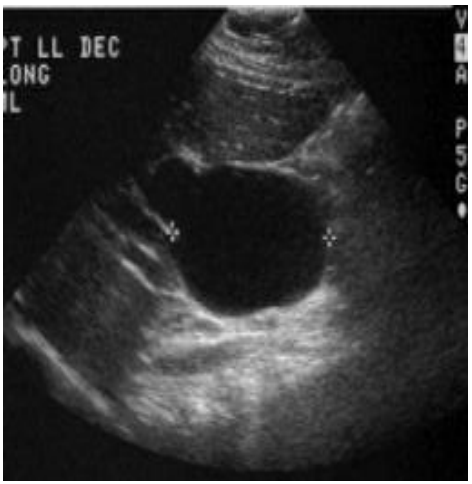

Fig. 2: US showing Choledocal cyst.

Idiopathic Neonatal Hepatitis

Idiopathic neonatal hepatitis, also known as giant cell hepatitis, accounts for approximately one-third of the cases of neonatal cholestasis. It is diagnosed by the presence of the classic pathological findings and the absence of any identifiable cause of cholestasis. There are two different categories: Sporadic cases and familial cases that could likely suggest an undiagnosed genetic or metabolic disease. These infants usually have low birth weight.

Jaundice is present within the first week of life. Acholic stools are usually absent unless there is severe cholestasis. On physical examination, liver is enlarged and firm in consistency. Serum bilirubin and transaminases are mildly elevated. Liver biopsy usually shows lobular disarray with hepatocellular swelling (ballooning), focal hepatic necrosis and giant cell transformation. Management is usually supportive with nutritional support, vitamin supplementation and treatment of complications of cholestasis. Prognosis is variable with sporadic cases having very good prognosis with 90% resolution by age 1 year and relatively poor prognosis in familial cases suggesting some inborn errors.[13]

Metabolic and Genetic Causes of Neonatal Cholestasis

Metabolic disorders present with a wide variety of clinical manifestations depending on the nature of underlying defect.

Recent progress in liver cell biology and molecular genetics has dramatically changed our understanding of the molecular pathogenesis of congenital and familial cholestatic diseases. The concept that familial cholestatic disorders are caused by mutations in genes coding for hepatobiliary transport proteins has nicely bridged basic and clinical medicine and will soon have an impact on our current diagnostic and clinical practice.[14]

Table (2) summarizes the metabolic and genetic causes of neonatal cholestasis, some of them will be discussed.

Table 2: Metabolic and Genetic Causes of Neonatal Cholestasis.[15]

Disease	Inborn error	Hepatic pathology	Diagnostic studies
Galactosemia	Galactose-1-phosphate uridylyltransferase deficiency	Cholestasis, steatosis, necrosis, pseudoacini, fibrosis	Galactose-1-phosphate uridylyltransferase assay of red blood cells
Fructosemia	Fructose-1-phosphate aldolase deficiency	Steatosis, necrosis, pseudoacini, fibrosis	Liver fructose-1-phosphate aldolase assay or leukocyte DNA analysis
Tyrosinemia	Fumarylacetoacetase deficiency	Necrosis, steatosis, pseudoacini, portal fibrosis	Urinary succinylacetone, fumarylacetoacetase assay of RBCs
Cystic fibrosis	Cystic fibrosis transmembrane regulator gene mutations	Cholestasis, neoductular proliferation, bile duct mucus, portal fibrosis	Sweat test and leukocyte DNA analysis
Hypopituitarism	Deficient production of pituitary hormones	Cholestasis, giant cells	Thyroxine, thyroid-stimulating hormone cortisol levels
∞1-Antitrypsin deficiency	∞1-Antitrypsin gene mutation (PiZZ phenotype)	steatosis,fibrosis Giant cells, cholestasis, neoductular proliferation	Serum ∞1-antitrypsin phenotype PAS diastase- resistant cytoplasmic granules
Gaucher's disease	b-Glucosidase deficiency	Cholestasis, HSM ytoplasmic inclusions Kupffer cells (foam cells)	b-Glucosidase assay in leukocytes
Niemann-Pick disease	Lysosomal sphingomyelinase deficiency	Cholestasis, cytoplasmic inclusions in Kupffer cells & HSM	Sphingomyelinase assay of leukocytes or liver or fibroblasts (type C); leukocyte DNA analysis
Glycogen storage disease type IV	Branching enzyme deficiency	Fibrosis, cirrhosis, PAS diastase-resistant cytoplasmic inclusions	Branching enzyme analysis of leukocytes or Liver
Neonatal iron storage disease	Unknown	Giant cells, portal fibrosis cirrhosis , hemosiderosis,	Histology, iron stains, buccal mucosa, biopsy
Peroxisomal disorders (e.g.Zellweger syndrome)	Deficient peroxisomal enzymes	Cholestasis, necrosis fibrosis, cirrhosis,	Plasma very-long-chain fatty acids, qualitative bile acids, pipecolic acid,liver electron microscopy
Abnormalities in bile acid metabolism	Several enzyme deficiencies defined	Cholestasis, necrosis, giant cells	Urine, serum duodenal fluid analyzed for bile acids by fast-atom bombardment mass spectroscopy
PFIC types 1 and 2	FIC1 and BSEP gene mutations	Cholestasis, necrosis, giant cells, fibrosis (pericentral)	Histology, family history low or normal GGT normal cholesterol DNA analysis
PFIC type 3	MDR-3 gene mutations	Cholestasis, bile duct proliferation, Portal fibrosis	Bile phospholipid level DNA analysis
Alagille's syndrome (syndromic paucity, of interlobular bile ducts)	Jagged 1 gene mutations	Cholestasis paucity of interlobular bile, ducts, increased copper levels,	Three or more clinical features liver histology DNA analysis

∞ 1-Antitrypsin Deficiency (∞ 1-AT):

This is the most common inherited cause of neonatal cholestasis.

∞1-AT is a protease inhibitor produced in the liver. The deficiency is caused by mutations in the gene found on chromosome 14. More than 75 different phenotypes of ∞1-AT are named according to migration characteristics on polyacrylamide gels, based on differences in isoelectric point (Pi), with M normal and Z most deficient. The incidence of homozygous PiZZ that is associated with neonatal liver disease and adult emphysema is 1 in 2000 live births in European and North American populations. Only 15% of PiZZ neonates develop clinical disease within the next 20 years. The mechanism of liver disease is accumulation of the defective molecule in the liver.

Clinical presentation is very similar to biliary atresia. These infants also have intrauterine growth retardation and are more likely to develop coagulopathy. Diagnosis is confirmed by documenting low plasma ∞1-AT levels and determining ∞1-AT phenotype.

Management is mostly supportive with nutritional supplementation. Prognosis is related to the severity of the liver disease. In children with progressive liver disease, liver transplantation has shown good survival rates of 90% at 1 year and 80% at 5 years. Prospects for therapy include attempts to block ∞1-AT accumulation in the liver or increase the turnover of the accumulated abnormal ∞ 1-AT protein.[16]

Progressive Familial Intrahepatic Cholestasis (PFIC):

PFIC is a group of genetic disorders that show progressive intrahepatic cholestasis. All these disorders have an autosomal recessive inheritance.

PFIC-1: Is caused by mutation in the FIC 1 gene mapped to chromosome 18q21-22 and is the original Byler disease described in the descendants of an Amish American family. The FIC1 gene is expressed in the canalicular membranes. Patients present with episodic cholestasis in the first month of life. Diarrhea, pancreatitis and deficiency of fat-soluble vitamins are seen. Serum gamma-glutamyl transpeptidase (GGT) levels are normal. Liver biopsy shows bile duct paucity. Electron microscopy shows granular appearance of bile present in the canaliculus. Management is mostly supportive. Surgical methods like ileal exclusion, partial external biliary diversion have been tried. Cirrhosis is seen by end of first decade of life and liver transplantation is needed with hepatic decompensation and is usually needed around the second decade of life.

PFIC-2: Is caused by a defect in the canalicular bile salt excretory pump (BSEP) which has a gene locus at chromosome 2q 24. Clinical presentation is similar to PFIC 1 except for the absence of pancreatitis in this condition. Liver biopsy shows more inflammation and electron microscopy shows amorphous bile. Management is again supportive. Prognosis is worse, with patients requiring liver transplantation in the first decade of life.

PFIC-3: Is caused by a defect in the canalicular phospholipids transporter, multiple drug resistant (MDR3) located on chromosome 7q21. Clinical presentation is similar to PFIC-1 but is delayed until early adulthood. There is a history of cholestasis of pregnancy in the mother. Gamma-glutamyl transpeptidase (GGT) is markedly elevated and bile analysis shows high bile acid to phospholipids ratio. Liver biopsy may mimic biliary atresia but the biliary tract is patent. Treatment is mostly supportive and prognosis is variable.[17]

Benign recurrent intrahepaticcholestasis (BRIC):

BRIC, or Summerskill-Walshe syndrome, has also been mapped to chromosomal region 18q21-22 and to mutations in the FIC 1 gene.

This disease is not rare and manifests with recurrent episodes of cholestasis, the first attack being possible at any age. Cholestasis usually ensues following a prodrome with flu-like syndrome and may last for several weeks. Laboratory tests show elevation of serum bile acids, cholesterol, alkaline phosphatase and transaminases, but gamma-glutamyl transpeptidase (GGT) is only slightly elevated. As in PFICl, serum lithocholic acid concentrations are substantially elevated. Histology is unremarkable, except for cholestasis.

There is a high degree of phenotypic variability, but progression of the liver disease does not occur.

FICl mutations described in BRIC patients include a missense mutation and a small deletion; unlike PFICl, mutations in BRIC patients are localized in less highly conserved regions of the FICl gene and likely to have a lower impact on structure and function of the transporter.[18]

Alagille Syndrome:

Alagille syndrome is an autosomal dominant disorder characterized by paucity of the interlobular bile ducts. The incidence is reported to be 1 in 100,000 births. It is also known as Watson-Alagille syndrome, arteriohepatic dysplasia, syndromic bile duct paucity (SBDP), syndromic intrahepatic biliary hypoplasia, and intrahepatic biliary dysgenesis. Alagille syndrome is caused by mutations in the human Jagged 1 gene that has been mapped to chromosome 20p12. This gene encodes a ligand for the Notch signaling pathway.[19]

Clinically, this syndrome is characterized by chronic cholestasis; characteristic facies with a broad forehead, small chin and saddle nose with bulbous tip and hypertelorism; skeletal anomalies including butterfly vertebrae, curved phalanges and short ulna; cardiac anomalies most commonly peripheral pulmonic stenosis and also including tetralogy of Fallot, pulmonary atresia, truncus arteriosus and VSD; and ocular anomalies like posterior embryotoxon and optic nerve drusen. Other findings include renal abnormalities like ectopic kidney, small kidneys, multicystic kidneys, renal artery stenosis; mental retardation and developmental delay; growth retardation and pancreatic insufficiency. Infants usually present with neonatal cholestasis. It may be difficult to differentiate from biliary atresia initially because in some cases initial liver biopsy may show bile duct proliferation.[20]

Management consists of aggressive nutritional and fat-soluble vitamin support. A major disabling feature for many children is pruritus. Pancreatic supplementation may be necessary. Liver transplantation may also be indicated in some children because of severe impairment of quality of life due to pruritus.[8]

Disorders of Bile Acid Synthesis

In 1984, infants with suspected neonatal cholestasis were screened for defects in bile acid synthesis using the-novel technique of fast-atom bombardment ionization mass spectrometry (FAB-MS). Using this approach, six defects in bile acid synthesis have been identified to date and account for 2-3% of cholestatic disorders of infancy and childhood.

In neonates, the presentation is similar to PFIC. Laboratory tests are variable, but all infants have atypical urinary bile acids identified by FAB-MS. Treatment with oral cholic acid, 250 mg/day, normalized biochemical parameters and reversed liver disease in most cases.[21]

Medical management of cholestasis

Medical management of cholestasis is mostly supportive and does not alter the natural course of the disease. It is aimed mostly at treating the complications of chronic cholestasis like pruritus, malabsorption and nutritional deficiencies and portal hypertension.

Table (3) summarizes the drugs used for medical management of Cholestasis.

CONCLUSION

Neonatal jaundice lasting greater than 2 weeks should be explained. Pale stools and dark or yellow urine are evidence of liver disease which should be urgently investigated. The neonatal hepatitis syndrome has many causes and a structured approach to investigation is mandatory. It should be possible to confirm or exclude biliary atresia so that, definitive surgery is not delayed unnecessarily. Infants with cholestasis should have vigorous fat-soluble vitamin supplementation including parenteral vitamin K if coagulation is abnormal.

Table 3: Symptomatic Treatment of Cholestasis.[15]

Indication	Treatment	Dose	Toxicity /comments
cholestasis :	- Phenobarbital	3-10 mg/kg/day	- Drowsiness, irritability, interference with vitamin D metabolism
	- Cholestyramine	250-500 mg/kg/day	- Constipation, acidosis, binding of drugs, increased steatorrhea
	- Ursodeoxycholic acid	15-20 mg/kg/day	-Transient increase in pruritus
Pruritus:	- Phenobarbital or cholestyramine (or both)		as above
	- Antihistamines		
	Diphenhydramine	5-10 mg/kg/day	- Drowsiness
	Hydrochloride hydroxyzine	2-5 mg/kg/day	- Drowsiness
	- Ultraviolet B light Exposure	as needed	- Skin burn
	- Carbamazepine	20-40 mg/kg/day	- Hepatotoxicity, marrow suppression, fluid retention
	- Rifampin	10 mg/kg/day	- Hepatotoxicity, marrow suppression
	- Ursodeoxycholic acid	15-20 mg/kg/day	-Transient increase in pruritus
Steatorrhea	- Formula containing medium-chain triglycerides (e.g., Pregestimil)	120-150 calories/kg/day for infants	- Expensive
	- Oil supplement containing medium-chain triglycerides	1-2 ml/kg/day	- Diarrhea, aspiration
Malabsorption of fat-soluble vitamins:	- Vitamin A	5,000-25.000 units/day	- Hepatitis, pseudotumor cerebri, bone lesions
	- Vitamin D	2000-6000 units/day	- Hypercalcemia, hypercalciuria
	25-Hydroxycholecalciferol (vitamin D)	3-5 mcg/kg/day	- Hypercalcemia, hypercalciuria
	1,25-Dihydroxycholecalciferol (vitamin D)	0.05-0.2 mcg/kg/day	- Hypercalcemia, hypercalciuria
	- Vitamin E (oral)	25-200 IU/kg/day	- Potentiation of vitamin K deficiency
	Vitamin E (TPGS,liqui	15-25 IU/kg/day	- Potentiation of vitamin K deficiency
	- Vitamin K (oral)	2.5 mg twice/wk to 5 mg/day	
Malabsorption of other nutrients:	- Multiple vitamins Up to twice the standard dose		
	- Calcium	25-100 mg/kg/day	- Hypercalcemia, hypercalciuria
	-Phosphorus	25-50 mg/kg/day	- Gastrointestinal intolerance
	- Zinc	1 mg/kg/day	- Interference with copper and iron absorption

REFERENCES

1. O'Connor JA, Sokol RJ. Neonatal cholestasis and biliary atresia. Encyclopedia of Gastroenterology 2004: 696-706.

2. Venigalla S, Gourley GR. Neonatal cholestasis. Semin. Perinatol. 2004;28 (5): 348-55.

3. Geier A, Fickert P, Trauner M. Mechanisms of disease: Mechanisms and clinical implications of cholestasis in sepsis. Nat. Clin. Pract. Gastroenterol. Hepatol. 2006;3 (10): 574-85.

4. Dellert SF, Balistreri WF. Neonatal cholestasis. In: Durie PR, Hamilton JR, Walker-Smith JA, Watkins JB, Walker WA, editors. Pediatric gastrointestinal disease: Pathophysiology, diagnosis and management. 3rd ed.: B.C. Decker; 2000. p. 890-4.

5. Balistreri WF. Intrahepatic cholestasis. J.Pediatr.Gastroenterol.Nutr. 2002; 35 Suppl 1:S17-23.

6. Suchy FJ. Approach to the infant with cholestasis. In: Suchy FJ, Sokol RJ, Balistreri WF, editors. Liver disease in children. 3rd ed.: Cambridge University Press; 2007. p. 179-89.

7. Moyer V, Freese DK, Whitington PF, Olson AD, Brewer F, Colletti RB, et al. Guideline for the evaluation of cholestatic jaundice in infants: Recommendations of the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. J.Pediatr. Gastroenterol. Nutr. 2004;39 (2):115-28.

8. McKiernan PJ. The infant with prolonged jaundice: Investigation and management. Curr. Paediatr. 2001;11 (2): 83-9.

9. Haber BA, Russo P. Biliary atresia. Gastroenterol.Clin.North Am. 2003; 32 (3): 891-911.

10. Sokol RJ, Mack C. Etiopathogenesis of biliary atresia. Semin. Liver Dis. 2001; 21 (4): 517-24.

11. McKiernan PJ. Neonatal cholestasis. Semin. Neonatol. 2002; 7 (2): 153-65.

12. McKiernan PJ, Baker AJ, Kelly DA. The frequency and outcome of biliary atresia in the UK and Ireland. Lancet 2000 1; 355 (9197): 25-9.

13. Roberts EA. Neonatal hepatitis syndrome. Semin. eonatol.2003; 8 (5): 357-74.

14. Colombo C, Okolicsanyi L, Strazzabosco M. Advances in familial and congenital cholestatic diseases. Clinical and diagnostic implications. Dig. Liver Dis. 2000; 32 (2):152-9.

15. Sokol RJ, Narkewicz MR. Liver and pancreas. In: Hay WW, Hayward AR, Levin mJ, Sondheimer JM, editors. Current pediatric diagnosis & treatment. 16th ed.: McGraw-Hill/Appleton & Lange; 2002. p. 647-92.

16. Carrell RW, Lomas DA. Alpha1-antitrypsin deficiency--a model for conformational diseases. N. Engl. J.Med. 2002 3; 346 (1): 45-53.

17. Jacquemin E. Progressive familial intrahepatic cholestasis. Genetic basis and treatment. Clin. Liver Dis. 2000; 4 (4): 753-63.

18. Suchy FJ, Shneider BL. Familial hepatocellular cholestasis. In: Suchy FJ, Sokol RJ, Balistreri WF, editors. Liver disease in children. 3rd ed.: Cambridge University Press; 2007. p. 310-25.

19. Warthen DM, Moore EC, Kamath BM, Morrissette JJ, Sanchez P, Piccoli DA, et al. Jagged1 (JAG1) mutations in Alagille syndrome: Increasing the mutation detection rate. Hum. Mutat. 2006; 27 (5): 436-43.

20. Arvay JL, Zemel BS, Gallagher PR, Rovner AJ, Mulberg AE, Stallings VA, et al. Body composition of children aged 1 to 12 years with biliary atresia or Alagille syndrome. J. Pediatr.Gastroenterol. Nutr. 2005;40 (2): 146-50.

21. Setchell KDR, O'Connell NC. Disorders of bile acid synthesis and metabolism: A metabolic basis for liver disease. In: Suchy FJ, Sokol RJ, Balistreri WF, editors. Liver disease in children. 2nd ed.: Lippincott Williams & Wilkins; 2001. p. 701-33.