Neonatal Cholestasis: A Pandora's Box

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ABSTRACT: Neonatal cholestasis (NC) is a diagnostic dilemma frequently countered in a neonatal care unit. Early diagnosis is vital for achieving an optimal patient outcome as many causes of cholestasis such as biliary atresia are time-sensitive and amenable to treatment if analyzed and treated early. Nonetheless, it is not generally simple to analyze these cases right on time as some of them are regularly missed due to the presence of pigmented stools, lack of newborn metabolic screening, and named as instances of prolonged jaundice. In this manner, we prescribe to explore all reasons for prolonged jaundice stretching out past 14 days in neonates. Besides, we suggest that stool card ought to be a piece of release rundown for all newborn children being released from the nursery. This is of most extreme significance in the nation like India where guaranteeing customary follow-up is as yet a tough assignment. These stool cards will help in the early determination of patients with NC particularly biliary atresia and guarantee their auspicious cure. Another reason which needs exceptional say is parenteral nutrition–associated liver illness, as the proportion of preterm babies is getting greater and greater with better neonatal care. These extreme preterm infants are in the requirement for prolonged (>14 days) total parenteral nourishment because of which they are at high hazard for NC contrasted with their more developed peers. In this survey, we will give an understanding of clinical approach, differential diagnosis, and clinical review of NC.

KEYWORDS: neonatal cholestasis, biliary atresia, hepatoportoenterostomy, liver biopsy, neonatal jaundice, radionuclide scan

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Definition and Epidemiology

The term cholestasis refers to diminished bile formation and/or excretion, and the term "neonatal cholestasis" is often used to refer to conjugated hyperbilirubinemia that is present either at birth or develops within the first few months of life. Conjugated hyperbilirubinemia is classically defined as conjugated bilirubin greater than 20% of total serum bilirubin with a minimum level of 1 mg/dL. Bile is formed in the liver and is a blend of bile acids, bilirubin, and fats. It is secreted into canaliculus; from that point, it flows into bile ducts and is at last discharged into the intestine after transient stockpiling inside the gallbladder. Alteration in the normal stream of bile brings about the unusual aggregation of bile salts, bilirubin, and lipids in liver and the blood. Despite the fact that direct hyperbilirubinemia and cholestasis are 2 distinct terms, the simple accessibility, minimal effort, low cost, and abnormal retention of conjugated bilirubin in neonatal cholestasis (NC) make it a reliable surrogate marker of cholestasis.[1–3] The overall incidence of NC is reported to be around 1 in 2500 live births. In India, NC constitutes approximately 30% of hepatobiliary disorders in India. By and large, a baby of NC presents at 4.5 weeks of age to a doctor for seeking therapeutic care in India and the average age of presentation to a tertiary center like ours in India is 3.5 months (range: birth to 15 months). Even in developed countries such as Germany and United States, the average age at diagnosis is 60 days. Furthermore, lack of adequate diagnostic facilities and expertise in diagnosing and managing such cases adds to the delay and leads to compromised patient care. All these factors taken together culminate in higher preventable morbidity and mortality related to NC. Even though high-performance liquid chromatography is viewed as the highest quality level, many centers across the world still use the diazo method for evaluating bilirubin, which tends to overestimate the direct fraction at lower bilirubin levels.[4–7]

Figure 1. Acholic stools due to inspissated bile syndrome in a 2-month-old neonate with Rh hemolytic disease.

Clinical Profile

Presentation of NC is protean, extending across yellowish discoloration of skin to acute liver failure and death. A thorough head-to-toe examination is a must for any case of NC. Acholic stools and high-colored urine are the characteristic terms used to describe an infant with cholestasis (Figure 1). Stools should always be pressed into a paste to obtain its true color. However, these features may not generally be available,

and an infant may likewise present with bleeding diathesis; pruritis; deficiency of vitamins A, D, E, K; and failure to thrive. Besides these general symptoms, there are specific clinical features depending on the cause. Coagulopathy may be caused by vitamin K deficiency, liver failure, or severe metabolic derangement of the liver (as in neonatal hemochromatosis). Splenomegaly can be seen in infants who have cirrhosis and portal hypertension, intrauterine infections, storage diseases, and hemolytic disorders such as Rh-isoimmunization. Neurologic abnormalities including irritability, lethargy, poor feeding, hypotonia, or seizures can indicate sepsis, intracranial hemorrhage, metabolic (including Zellweger syndrome) and mitochondrial disorders, or severe liver dysfunction resulting in hyperammonemia and encephalopathy. Low birth weight, thrombocytopenia, petechiae and purpura, and chorioretinitis are regularly associated with intrauterine infections. Congenital infections may also be associated with rash, microcephaly, intrauterine growth restriction, and intracranial calcification. Facial dysmorphism may suggest a chromosomal abnormality or Alagille syndrome. An obvious mass in the upper quadrant of the abdomen may indicate a choledochal cyst. A cardiac murmur increases the likelihood of Alagille syndrome or biliary atresia. On physical examination, infants with biliary atresia are generally thriving well and are appearing well except for jaundice, and stools are often acholic. However, biliary atresia may present with features of advanced liver disease such as ascites and hepatosplenomegaly if there is a delay in diagnosis. By contrast, infants with infectious and metabolic cause appear sick and have inadequate weight gain.[8] A peculiar odor of body or urine may point to a metabolic cause. Examination of male genitalia and ability to fix and follow a moving object may be useful clues for panhypopituitarism and septo-optic dysplasia, respectively.

Etiology

A myriad of potential causes have been identified causing NC and this list is ever increasing with improvement and upgradation of diagnostics and laboratory services. Causes of NC can be broadly divided into surgical and medical causes. Overall biliary atresia is the most common identifiable cause of NC.[7–14] According to a latest published meta-analysis comprising 17 studies encompassing 1692 infants, idiopathic neonatal hepatitis was observed in 26%, extrahepatic biliary atresia in 26%, infection in 12%, TPN-associated cholestasis in 6.5%, metabolic disease in 4.37%, α_1-antitrypsin deficiency in 4.14%, and perinatal hypoxia/ischemia in 4% of infants with NC. Among the infections and metabolic disorders, cytomegalovirus (CMV) and galactosemia (37% of metabolic disorders) were the most common identifiable causes, respectively.[11] Lack of uniform protocol for diagnosing NC was the main drawback of study (Table 1).

Diagnostic Approach

Joint recommendation by the North American Society for Pediatric Gastroenterology,[3,8,15–35] Hepatology, and Nutrition and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition 2016, has defined

> abnormal direct/conjugated bilirubin as a serum value >1.0 mg/dL (17 mmol/L), because it is physiologically and clinically complex to consider incorporating consideration of whether or not the direct fraction exceeds 20% of the total bilirubin level as mentioned in some publications.[14]

In cases of preterm and small for gestation, with a negative septic and metabolic screen and a normal ultrasound abdomen, a close observation period till the corrected age of 40 weeks or weight of 2.5 kg can be considered, respectively. Commonly employed investigations in a patient of NC are given in Table 2. The aim of the evaluation is to look for treatable causes at the earliest and start specific treatment as appropriate depending on history and clinical evaluation. In general, hepatocellular etiology or hepatitis is associated with a rise in liver enzymes and extrahepatic causes are associated with rise in γ-glutamyl transpeptidase with few exceptions. To access the hepatocellular damage, one needs to do a serum albumin and coagulation profile (prothrombin time, international normalized ratio). Common treatable causes of NC include sepsis (bacterial; viral; CMV, HSV; fungal; etc), thyroid disorder, galactosemia, and hemochromatosis.

We provide a schematic diagram to work up a patient of NC syndrome in Figure 2. All patients with age more than 60 days who have previously been admitted and have still not being diagnosed should be referred to a tertiary center with facility for liver transplant for optimal management.

Role of Genomics in the evaluation of NC: Genetic Cholestasis

Use of genomics especially whole-genome sequencing (WGS/WES) and next-generation sequencing has an increasing role in the diagnosis of rare disorders in ill neonates and has the potential to augment or modify the care of this vulnerable population of patients.[36] However, cost, interpretation of results and availability are the major obstacles in ordering these investigations. Nevertheless, genomics has made the biggest impact in neonatology than any other specialties of medicine. In addition, the advantage of diagnosis in antenatal period cannot be ignored. Keeping the above constrains in mind, targeted genomics is the need of the hour. Targeted genetic testing represents a multitiered approach offered either presymptomatically or postsymptomatically. Presymptomatic testing is offered to individuals with a known genetic disorder in the family, whereas postsymptomatic genetic testing should be offered to individuals suspected clinically to be affected by genetic disease, ranging from gene specific to panel specific to WGS/WES.[37] Some of the disorders which can be unveiled with

Table 1. Causes of neonatal cholestasis.

SURGICAL (EXTRAHEPATIC)	MEDICAL (HEPATOCELLULAR)
• Biliary atresia (EHBA) (26%) • Choledochal cyst • Cholelithiasis • Inspissated bile secretion • Spontaneous perforation of the bile duct • Nonsyndromic paucity of bile ducts • Congenital hepatic fibrosis/Caroli disease • Neonatal sclerosing cholangitis	❖ Idiopathic neonatal hepatitis (26%) ❖ Prematurity (10%) ❖ Small for gestation (SGA) ❖ Infections (12%) ❖ Viral: CMV (32%) TORCH, HIV, echovirus, Adenovirus, coxsackie virus, HBV, rubella, reovirus, adenovirus, human herpes virus 6, varicella zoster, herpes simplex, parvovirus, hepatitis B and C, human immunodeficiency virus ❖ Bacterial (sepsis, urinary tract infection, syphilis, tuberculosis) ❖ Scrub typhus ❖ Intrauterine infections (5%) • Endocrine disorders ❖ Hypothyroidism/hyperthyroidism ❖ Hypopituitarism ❖ Cortisol deficiency • Genetic/metabolic (20%) ❖ Galactosemia (37%) ❖ Alagille syndrome (2%-6%) ❖ Cystic fibrosis ❖ α_1-antitrypsin deficiency (5%-15%) (not reported in India) ❖ Indian childhood cirrhosis ❖ Progressive familial intrahepatic cholestasis (PFIC 10%) ❖ Tyrosinemia ❖ Fructosemia ❖ Inborn errors of bile acid metabolism ❖ Neonatal hemochromatosis ❖ Gaucher disease ❖ Wolman disease ❖ Niemann-Pick type C ❖ Mitochondrial disorders ❖ Congenital disorders of glycosylation ❖ Peroxisomal disorders ❖ Dubin-Johnson and Rotor syndrome • Chromosomal disorders ❖ Trisomy 21, 13, 18 ❖ Turner syndrome ❖ Cat eye syndrome • Systemic disorders ❖ Shock, heart failure, asphyxia (4%) • Miscellaneous ❖ Hemophagocytic lymphohistiocytosis (HLH) ❖ Neonatal leukemia ❖ Erythroblastosis fetalis ❖ "Le foie vide" (infantile hepatic nonregenerative disorder) ❖ Neonatal lupus erythematous ❖ Prolonged TPN (6.5%) ❖ ARC syndrome (arthrogryposis, renal tubular dysfunction, and cholestasis)

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Table 2. Diagnostic workup for neonatal cholestasis.

INVESTIGATION	SALIENT FEATURES
1. Conjugated bilirubin • Diazo/van den Bergh method reports direct bilirubin, which includes both conjugated bilirubin and delta bilirubin (conjugated bilirubin with albumin)	Should be >20% of total bilirubin
2. Alanine aminotransferase (ALT), aspartate aminotransferase (AST)	Nonspecific for etiology, rise more than twice the normal levels suggestive of hepatocellular injury • AST is also present in red cells and myocytes
3. PT/APTT and INR >3	Depicts severity, pathognomic of acute liver failure
4. Alkaline phosphatase (less specific than GGT)	Can rise in hepatobiliary, kidney or bone disease, usually elevated in infants

(Continued)

Table 2. (Continued)

INVESTIGATION	SALIENT FEATURES
5. γ-Glutamyl transpeptidase (GGT) (*Higher in neonates than older children*)	• ↑ in biliary atresia, α_1-antitrypsin deficiency, progressive familial intrahepatic cholestasis PFIC type 3 (due to ABCB4 deficiency), Alagille syndrome and idiopathic neonatal hepatitis • Normal or decreased in progressive familial intrahepatic cholestasis (PFIC) type 1 (ATP8B1 deficiency) and 2 (ABCB11 deficiency), bile acid synthesis disorders (BASDs), and tight-junction protein (TJP) type 2 deficiency
6. Sepsis screen, urine, blood, and CSF cultures	• Sepsis screen, urine, blood, and CSF cultures
7. Fasting and post feeding USG abdomen ➢ Neuro-sonogram ➢ Color Doppler for hepatic artery diameter, and hepatic artery diameter to portal vein diameter ratio, subcapsular blood flow (*A normal ultrasound does not rule our biliary atresia*)	• Triangular cord sign (triangular echogenic area due to fibrous tissue at the porta hepatis), small (<15 mm) or nonvisualization of the gallbladder, lack of gallbladder contraction after oral feeding are suggestive of biliary atresia • To establish diagnosis of choledochal cyst • To look for complications such as ascites, portal hypertension, intraventricular bleed, and associated malformation like polysplenia
8. Urine for reducing substances and galactose-1-phosphate uridylyltransferase levels	• Measured by spectrophotometry (normal levels: 11-41 U/g Hb)
9. Hepatobiliary scintigraphy using Technetium-99m-labeled iminodiacetic acid (HIDA scan) ➢ Sensitivity 98.7% ➢ Specificity 70.4% ➢ Single photon emission computer tomography (SPECT) can improve the specificity (81.1%) ➢ Gastrointestinal and duodenal fluid sampling increases the specificity to 73.2% and 77.1%, respectively ➢ High rates of false positive in interlobular bile duct paucity, idiopathic neonatal hepatitis, low birth weight, and those on total parenteral nutrition neonates	• Requires controversial pretreatment with phenobarbitone (5 mg/kg/d) for at least 3-5 days for best results. Serial images to be taken for up to 24 h or until the gut activity are visualized. Suggestive of biliary atresia or severe hepatocellular dysfunction if there is nonvisualization of the radioisotope in the small intestine. However, if there is a slow uptake of the radioisotope or nonvisualization of the liver, it suggests hepatocellular dysfunction • Only a supportive investigation should only be done when available in the same center, otherwise should not be done as it may result in wastage of crucial time, money, and resources • Best use is to exclude biliary atresia
10. Liver biopsy for diagnosis and prognosis depending on the extent of fibrosis ➢ Sensitivity for biliary atresia: >95% ➢ Specificity for biliary atresia >94% ➢ Features suggestive of biliary atresia include ✓ bile duct proliferation, bile plugs ✓ Portal tract edema and fibrosis ✓ with preservation of the basic hepatic lobular architecture	• Useful for biliary atresia, α_1-antitrypsin deficiency, Alagille syndrome, neonatal sclerosing cholangitis, cytomegalovirus, and herpes simplex infections ➢ Avoid early biopsy, may lead to false-negative results, repeat when in doubt ➢ Histologic mimics of biliary atresia include cystic fibrosis, PNALD, and α_1-antitrypsin deficiency
11. α_1-antitrypsin phenotype (Pi typing) and level ➢ PI analysis (type ZZ, SZ, MZ)	Levels are usually confusing with high false-positive and false-negative rates. Pi testing is useful
12. X-ray chest and spine	To look for associated anomalies associated with biliary atresia and Alagille syndrome (20%-30%) especially in the presence of a murmur
13. Alpha-feto (AFP) protein levels and urine for succinyl acetone	• AFP levels although nonspecific are markedly raised in tyrosinemia • Urine for succinyl acetone should be done in a freshly passed urine sample. Delay in processing may increase false-negative rates
14. PCR (blood and/or CSF)	• CMV, HSV, Listeria
15. Sweat chloride analysis/serum immunoreactive trypsinogen (IRT) level/CFTR genetic testing	• For cystic fibrosis
16. Magnetic resonance cholangiography (MRC) ➢ Endoscopic retrograde cholangiography (ERC) ➢ Sensitivity 86%-100%, specificity 87%-94% (*Positive predictive value 88%-96%, negative predictive value 100%*)	• Still an evolving technique. Lack of clear-cut data, small probes, and need for general anesthesia precluding their use
17. Echocardiography	• To look for associated cardiac lesion especially in biliary atresia and Alagille syndrome
18. Intraoperative cholangiogram ➢ False-positive rate: 20%	• Remains the gold standard test. However, testing for Alagille syndrome and cystic fibrosis before the procedure will decrease false-positive results
19. Acid esterase (lipase)	• For Wolman disease • Normal levels: 12-43.8 nmol/h/mg
20. Bone marrow aspiration	• For HLH, leukemia, metabolic diseases

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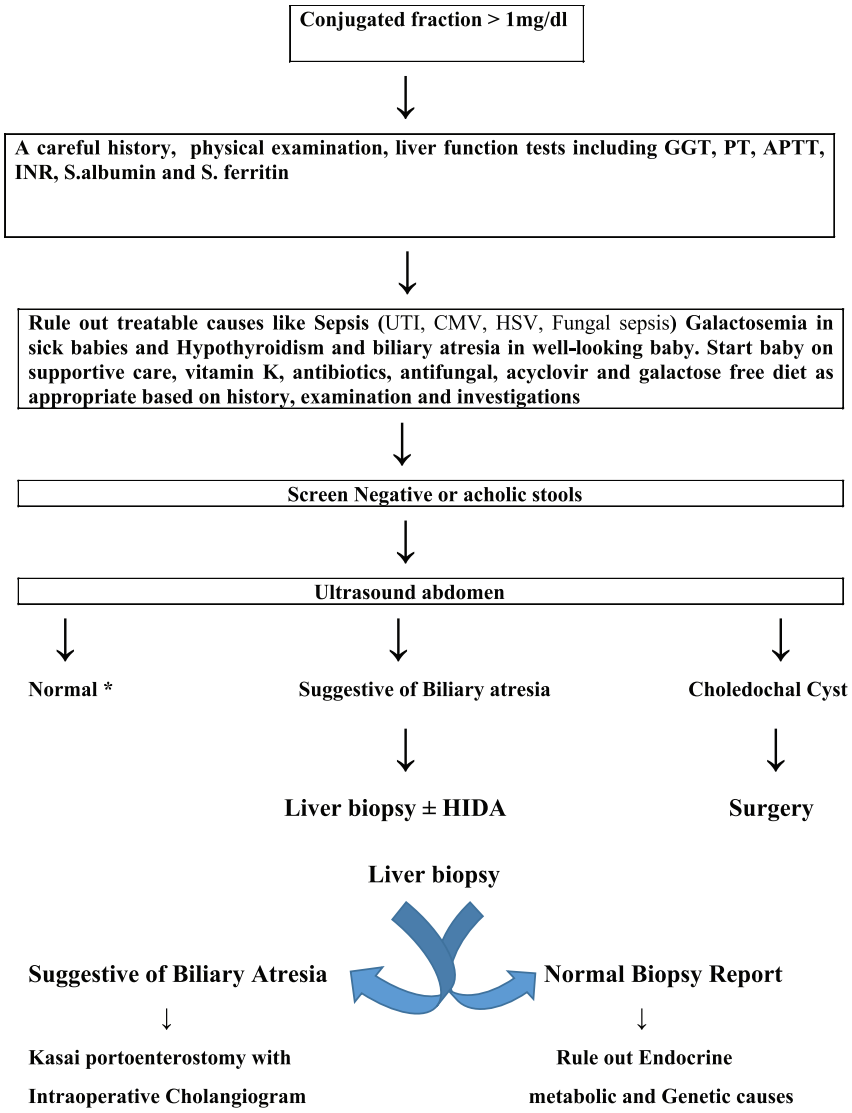

Figure 2. A schematic diagram to work up a patient with neonatal cholestasis syndrome.
*Workup includes sweat chloride, Pi typing, serum cortisol, urine GCMS, TMS, acyl carnitine profile, ANA, anti-Ro/SSA, anti-La/SSB, anti-U1RNP antibodies, urinary bile acid analysis, genetic analysis for PFIC (↑ liver enzymes and ↓ GGT) and bone marrow for hemophagocytic lymphohistiocytosis (HLH). Diagnostic criteria for HLH includes fever (>7 days), hepatosplenomegaly with liver dysfunction, pancytopenia, sCD25 (>2400 μg/mL), ferritin (>500 μg/L), triglycerides (>3 mmol/L), hypofibrinogenemia (<150 mg/dL), and serum cytokine levels of both IFNγ (>75 pg/mL) and IL-10 (>60 pg/mL) ↑.[36,37,42,43]

genomic testing are neonatal intrahepatic cholestasis caused by citrin deficiency (mutations in SLC25A13),[38] progressive familial intrahepatic cholestasis[39](mutations in either ATP8B1 or ABCB11), Alagille syndrome (mutations in either JAG1 or NOTCH2), Alagille syndrome and Dubin-Johnson syndrome (mutations in ABCC2). In a recent study by Togawa et al, they analyzed 109 Japanese infants with cholestasis and made a molecular genetic diagnosis in 26% of their cohort (28/109). In addition, 7.7% categorized clinically as of unknown cause were successfully categorized by the molecular genetic diagnosis.[40] In a similar report, Liu et al[41] reported the use of jaundice chip, which includes 5 disease-causing genes: SERPINA1, JAG1, ATP8B1, ABCB11, and ABCB4. With more and more research, we expect the percentage of patients diagnosed as genetic cholestasis to increase and a corresponding decrease in the unknown cause group. At present, we recommend a targeted genomic diagnosis in patients with NC especially those with atypical features.

Conclusions

Neonatal cholestasis must be considered in any infant presenting with prolonged jaundice longer than 2 weeks or early if associated with hepatomegaly, failure to thrive, acholic stools, or dark urine. Neonatal cholestasis, although rare, is a life-threatening condition unless timely diagnosed and managed appropriately. However, delay in diagnosis of NC, particularly of biliary atresia, remains a problem. Misinterpretation with physiological jaundice, lack of national-level screening programs for inborn errors of metabolism, and presence of pigmented stools are often the cause of delay in diagnosis. Universal screening for NC still has a long way to go. There is a need for strict follow-up, use of stool card, and awareness.

Even if there is no specific management, appropriate supportive care and nutritional rehabilitation may be life-saving in most cases of NC.

Author Contributions

AP wrote the initial manuscript, VG helped in literature search, GG and AP did the critical appraisal.

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