A Study on Early Onset Conjugated Hyperbilirubinemia in Neonates in a Tertiary Care Centre in Bhagalpur.

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ABSTRACT **Objectives :** To determine the related risk factors, outcomes of early onset conjugated hyperbilirubinemia in a group of newborn infants and also to determine the incidence of sepsis in these neonates.

Methods : 84 newborn infants with conjugated hyperbilirubinemia were retrospectively reviewed.

Results : The mean gestational age was 37 weeks, and the mean postnatal age at presentation was 10 days. Blood culture-proven sepsis was identified in 30 babies (35.7% of total). Gram negative bacteria were isolated in 20 cases and E. coli was the most common of these agents (14 cases). Perinatal hypoxia ischemia was the second most frequent etiology (14 patients; 16.7% of total). The other diagnoses were blood group incompatibility (n=10), Down syndrome (n=6), cholestasis associated with parenteral nutrition (n=6), neonatal hepatitis (n=4), metabolic liver disease (n=2), biliary atresia (n=2), portal venous thrombosis (n=2), and unknown (n=8). 26 babies with sepsis recovered completely with treatment, whereas the prognosis for those with perinatal hypoxia-ischemia was very bad. (12 patients out of 14 died).

Conclusion : From our study we concluded that early onset cholestatic jaundice in newborn infants is more commonly due to non-hepatic causes like neonatal sepsis and perinatal hypoxia. So it is very important to observe these infants carefully over a period of time before undertaking time consuming or invasive methods to investigate the primary liver disease. There is a felt need for early diagnosis by Pediatricians and other specialists who play a major role in identifying newborn infants with the risk of infantile conjugated hyperbilirubinemia and referring them for intervention at the earliest to bring down the menace of this disease.

KEYWORDS : Newborn; Conjugated hyperbilirubinemia; Sepsis; Perinatal hypoxia-ischemia.

INTRODUCTION

Jaundice refers to yellow discoloration of the skin, sclera, mucous membranes, and body fluids. It is a common problem that can be the presenting sign for many disorders. The challenge for the physician is to identify patients who need additional evaluation. In the newborn period and in early infancy, cholestatic jaundice, or conjugated hyperbilirubinemia, results from hepatobiliary dysfunction. The prevalence of this disorder is estimated at 1 out of every 2500 live births; extrahepatic biliary atresia (EHBA) and idiopathic neonatal hepatitis (INH) account for two thirds of cases of infantile cholestatic jaundice [1]. Jaundice is one of the most common clinical conditions in neonatal medicine. The majority of cases of unconjugated hyperbilirubinemia involve nonpathological causal factors, whereas cholestatic jaundice usually reflects serious underlying pathologies. [2,3] Some cases of neonatal cholestasis require immediate, specific medical or surgical treatment, so it is vital to apidly identify the cause. Infectious causes are usually the first to be investigated. Viral, bacterial, and parasitic infections can all cause neonatal cholestasis, but bacterial disease is the most frequent underlying problem. Such infections deserve special emphasis because neonates can present with cholestasis as a component of sepsis, and cholestasis may be the only clinical sign of infection in such cases.

This study aimed to determine the related risk factors, its outcome, and also to determine the incidence of sepsis in a group of newborn infants admitted for early onset conjugated hyperbilirubinemia in neonatal intensive care unit of Pediatrics Department, Jawaharlal Nehru Medical College and Hospital, Bhagalpur.

MATERIALS AND METHODS

The present study included 84 babies with conjugated hyperbilirubinemia who were admitted to the neonatal intensive care unit of Pediatrics Department, Jawaharlal Nehru Medical College and Hospital, Bhagalpur between February 2015 and March 20115 before the age of one month. Cholestasis was defined by the presence of jaundice associated with increased serum conjugated bilirubin levels (direct bilirubin fraction greater than 15% of total) and/or elevations in biliary enzymes (gamma glutamyl transpeptidase [GGT] or alkaline phosphatase [ALP]) or transaminases (aspartate transaminase [AST] or alanine transaminase [ALT]). The clinical and laboratory data in each patient's chart were retrospectively reviewed.

In each case, serum concentrations of total and conjugated bilirubin, AST, ALT, ALP, GGT, hemoglobin, and white blood cell and platelet counts were determined using standard laboratory methods. Sepsis was diagnosed strictly on the basis of a positive blood culture. In 34 cases, serum samples were analyzed for antibodies to TORCH (Toxoplasma, rubella, cytomegalovirus, herpes virus types I and II) infections. 36 infants were subjected to investigations for metabolic disease. Ultrasonography was performed in 44 of these cases; hepatobiliary scintigraphy with 99mTc diethyl-3-iodo-HIDA was performed in 18 cases; and liver biopsy was performed in 8 cases. Data were analyzed using the two-tailed t test. P values below 0.05 were considered statistically significant.

RESULTS

The mean and median gestational ages of the 84 infants were 37 and 39 weeks (range, 30 to 40 weeks) respectively; 30 infants had a gestational age below 37 weeks. The mean postnatal age at presentation was 10 days (range, 1 to 29 days). The mean (SD) peak serum levels of total and conjugated bilirubin were 292.4 (193.2) µmol/L (range, 44.9-877.2 µmol/L) and 129.9 (129.9) µmol/L (range, 29.1-629.3 µmol/L), respectively. The mean total bilirubin levels in the subgroup with sepsis was 413.8 µmol/L, and this was significantly higher than the mean level for all other patients combined (P=0.003). The mean levels of ALT and AST in the perinatal hypoxia-ischemia group were 259.7 U/L and 899.8 U/L, respectively, and both these were significantly higher than the levels in all other patients combined (P=0.029 and P=0.016, respectively). The frequencies of different diagnoses in the 84 cases are shown in **table 1.**

Culture-proven sepsis was identified in 30 babies (35.7%), and 110 of these infants had meningitis. Gram-negative bacteria were isolated in 20 of the 30 sepsis cases, and were the most frequently identified causal agents of cholestasis. These micro-organisms were Escherichia coli (n=14), Klebsiella pneumoniae (n=2), Pseudomonas aeruginosa (n=2), and Enterobacter aerogenes (n=2). The other micro-organisms isolated were Staphylococcus aureus (n=6) and coagulase-negative Staphylococcus spp. (n=4). Four of the 30 septic infants died, and all four had E. coli sepsis with meningitis. The remaining 26 babies recovered completely with treatment, and their bilirubin levels returned to normal.

TABLE 1. Frequencies of Different Diagnoses in the 84 Cases.

Diagnosis	No. (%)
Culture-proven sepsis	30 (35.7)
Perinatal hypoxia-ischemia	14 (16.7)
Blood group incompatibility	10 (11.9)

Trisomy 21	6 (7.1)
Total Parenteral Nutrition associated cholestasis	6 (7.1)
Neonatal hepatitis	4 (4.8)
Metabolic liver disease	2 (2.4)
Biliary atresia	2 (2.4)
Portal venous thrombosis	2 (2.4)
Unknown	8 (9.5)

Perinatal hypoxia-ischemia was the second most common reason for cholestasis in the 84 cases (14 patients; 16.7%). The prognosis for this subgroup was very bad, and 12 of the infants died due to multi organ failure.

Blood group incompatibility was diagnosed in 10 cases. Six of these neonates had ABO incompatibility, two had Rh incompatibility, and two had minor blood group incompatibility. An exchange transfusion was performed in four cases. Two patients with hydrops died and kernicterus developed in the other two. Two infants had metabolic liver disease and was diagnosed with galactosemia. The serum conjugated bilirubin level in these infant returned to normal, and follow-up revealed a favorable outcome. Two of the six patients with Down syndrome was diagnosed with cholestatic hepatitis based on liver biopsy, and the other two had hypothyroidism. No other associated factor was detected in the other two cases. 4 patients had neonatal hepatitis. Cytomegalovirus was detected in two cases, and no etiologic agent was found in the other two. On follow-up, all four patients' clinical status and biochemical values returned to normal. Biliary atresia was diagnosed in two infants, but both died five months after undergoing surgical treatment (Kasia"s operation).

DISCUSSION
As it is evident that there are many causes of cholestasis in young newborn infants, so early diagnosis and initiation of appropriate therapy is important, because the effects of this disorder are usually very serious. In our study, bacterial infection, and gram negative sepsis in particular, was the main cause of acute cholestasis in the 84 newborn infants studied. E. coli was the most common gram-negative agent isolated.

In a previous report that investigated etiologic factors in neonatal cholestasis, extrahepatic biliary atresia was diagnosed in 35% of 85 patients of mean age 61 days.[2] In a study that examined cholestatic jaundice in 36 infants (mean age of onset 40 days) and that excluded babies with sepsis, congenital infection was identified as the most common cause (38.8% of the cases).[4] The babies in both these series were older than the infants in our study, indicating that these previous reports were focused on chronic cholestasis. Another research group assessed 92 infants in which onset of jaundice occurred at a mean age of 7 days.[5] They found that, in most of these neonates, the transient cholestasis was caused by several associated factors, including immaturity of bile secretion and perinatal disease leading to hepatic hypoxia or ischemia. Previous series and our study findings reveal that the causes of early, acute cholestasis are different from those of chronic forms.

The neonatal liver does not function at the same level as the liver of an older child. This is due to the small pool of bile acids, limited bile acid synthesis, and immaturity of hepatobiliary transport function.[3] Such features make the neonatal liver physiologically more susceptible to cholestasis.[3,5] This early-onset cholestasis often develops by the end of the first week of life and is always transient.

Several studies have evaluated relationships between infection and hyperbilirubinemia. One investigation involved 93 newborns younger than 7 days old who had unexplained unconjugated bilirubinemia (levels higher than 17.1 μmol/L).[6] Three of these infants had positive blood cultures, and the identified micro-organisms were Proteus mirabilis, a Bacteroides species, and K. pneumoniae. [6] However, in a study of 306 newborns 21 days old or younger who had unconjugated hyperbilirubinemia, no cases of sepsis were detected.[7] Another report documented urinary tract infection in 7.5% of 160 asymptomatic infants with jaundice who were younger than 8 weeks old.[8] Two of the 12 infants who had urine cultures positive for K. pneumoniae and E. coli had higherthan-normal conjugated bilirubin fractions.[8] The infants in the above studies were otherwise healthy babies particularly with unconjugated hyperbilirubinemia, whereas the neonates in our study were seriously ill babies with high conjugated bilirubin levels. This could explain the higher incidence of sepsis in our population.

In an another study of 22 newborns with bacterial infection and hyperbilirubinemia, 13 infants were clinically ill, and some of these ill newborns had exceptionally high serum total bilirubin levels with high conjugated fractions.[9] In our investigation, the mean total bilirubin levels in the 30 newborns with sepsis were significantly higher than the mean levels in the other 56 patients. Eight of the 30 septic patients had extremely high total bilirubin levels (range, 538.6 to 877.2 μmol/L). One report on premature infants with gram-negative bacteremia noted that liver enzyme abnormalities were more frequent than elevated conjugated bilirubin levels.[10] However, in our study, only six of our septic patients had elevated liver enzymes and all six had E coli sepsis.

Infants with hyperbilirubinemia associated with infection may have elevated unconjugated bilirubin due to hemolysis, or elevated conjugated bilirubin due to cholestasis. Not all cases of cholestasis associated with sepsis feature direct infection of the liver. In sepsis without direct hepatic infection, bile flow becomes markedly reduced, leading to cholestasis and conjugated hyperbilirubinemia but no significant transaminase elevation. The mechanism of cholestasis in such cases is not clear, but some have been postulated, including changes in hepatic microcirculation, direct effects from bacterial products, and effects caused by endotoxin induced mediators.[3] Impairment of biliary excretory function and biliary stasis induced by E coli endotoxins has been demonstrated in animal studies.[11] As well, cholestasis is considered to be a consequence of the hepatocyte response to sepsis-associated cytokines. It is likely that such cytokines exacerbate the normal physiologic cholestasis in neonates, causing more severe cholestasis. It also has been theorized that mild hemolysis caused by micro-organisms can overload the neonatal liver's immature conjugating mechanism, leading to increased serum bilirubin levels. Parenteral nutrition also has been found to be associated with cholestasis and sepsis.[12,13]

We detected in our study that the second most important cause of conjugated hyperbilirubinemia was perinatal hypoxia-ischemia. Several other studies have also identified this as an important causal factor in transient neonatal cholestasis. [5,14] Bile secretion processes, which are already underdeveloped in neonates, can be further impaired by hepatic hypoxia-ischemia. The cellular mechanisms involved in bile secretion require energy. During hepatic hypoxia-ischemia, the level of adenosine triphosphate in hepatocytes decreases and bile flow diminishes concomitantly. Aside from energy depletion, damage to the biliary canaliculae and alteration of bile ducts during hepatic hypoxia-ischemia are other postulated mechanisms for conjugated hyperbilirubinemia. In our series, the significantly elevated mean levels of AST and ALT in our fourteen patients with perinatal hypoxia-ischemia were considered important signals of extrahepatic insult. Isolated perinatal hypoxia-ischemia should be considered a possible precursor of early neonatal cholestasis in newborns who have suffered no other predisposing insults to the liver, and in those with extremely high serum AST and ALT concentrations.

CONCLUSION
The etiologies of infantile conjugated hyperbilirubinemia are numerous. While specific symptoms may narrow the diagnostic possibilities, a complete history and physical examination, diagnostic imaging and laboratory investigations directed at the more common etiologies is required to make a prompt, definitive diagnosis. It is very crucial to identify the cause of neonatal cholestasis because many of these infants need prompt, focused and complete medical or surgical therapy. The causal factors in acute neonatal cholestasis are different from those involved in the chronic form of this condition, and early onset neonatal cholestasis is usually transient. Our findings in neonates with conjugated hyperbilirubinemia suggest that early onset cholestatic jaundice in newborn infants is more commonly from non-hepatic causes and transient, so it is reasonable to monitor these infants carefully for a period of time before undertaking time consuming or invasive investigations towards a primary liver disease. There is a felt need for early diagnosis by Pediatricians and other specialists who play a major role in identifying newborn infants with the risk of infantile conjugated hyperbilirubinemia and referring them for intervention at the earliest to bring down the menace of this disease.

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