Risk factors for neonatal cholestasis in small gestational age infants: case report and literature review

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CASE REPORTS

ABSTRACT

Cholestatic jaundice, defined as conjugated bilirubin levels higher than 1 mg/dl, is a bile formation and excretion disturbance. Its incidence is estimated at 1 case in 2500 live births, being 100-200 times higher in preterm infants less than 28 weeks gestational age. It occurs in biliary atresia, infectious diseases, endocrine and inherited metabolic diseases, Alagille Syndrome, preterm and intrauterine growth-restricted newborns, lack of enteral feeding, and is more frequent in male gender. It is a frequent complication of parenteral nutrition. In preterm and small for gestational age (SGA) infants, the etiology of cholestasis is multifactorial. Early diagnosis enables early therapeutic intervention.

We report the case of an SGA male preterm infant. He was born by C-section for fetal distress at 30 weeks gestational age with an 800 g birth weight. He developed mild respiratory distress, hypoglycemia, meconium ileus, and early cholestasis. Complex hematological, serological, and immunological tests were carried out; ultrasound evaluations were performed. The final diagnosis was cholestasis of multifactorial etiology in a preterm infant with severe intrauterine growth restriction (IUGR). Treatment with ursodeoxycholic acid was started. Three months later, the transaminases, bilirubin, gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP) triglycerides returned to normal.

Conclusion. Cholestasis in SGA infants is a severe condition potentially associated with life-threatening complications, requiring complex diagnostic evaluation.

Keywords: neonatal cholestasis, conjugated hyperbilirubinemia, cystic fibrosis, IUGR, preterm infant

Abbreviations (in alphabetical order):

AGA – appropriate for gestational age
ALP – alkaline phosphatase
ALT – alanine aminotransferase
ASP – aspartate aminotransferase
DHA – docosahexaenoic acid
ELBW – extremely low birth weight
EPA – eicosapentaenoic acid
GGT – gamma-glutamyl transferase
IUGR – intrauterine growth restriction
NEC – necrotizing enterocolitis
NICU – neonatal intensive care unit
SGA – small for gestational age
TPN – total parenteral nutrition
TSB – total serum bilirubin
WBC – white blood cells

INTRODUCTION

Neonatal cholestasis represents the alteration of the bile formation process at the level of hepatocytes and/or its clearance at the intra- or extrahepatic bile duct level, leading to biliary acid retention. It is defined as a conjugated bilirubin level of more...
than 1 mg/dl when the total serum bilirubin (TSB) level is 5 mg/dl or less or a conjugated bilirubin level of more than 20% of TSB level when TSB level is above 5 mg/dl, associated or not with increased levels of gamma-glutamyl transferase (GGT) and alkaline phosphatase (ALP)[1]. Many conditions cause neonatal cholestasis syndrome, its etiological diagnosis being a challenge, especially in preterm neonates.

The estimated incidence of neonatal cholestasis syndrome is 1 case in 2500 live newborns; it is higher in preterm and small for gestational age (SGA) infants, in whom it reaches 1 case in 200 live newborns. The incidence increases with lower gestational ages, so that in preterm babies below 30 gestational weeks, it is 14.8%, and the mortality rate is 13.5% [2].

Classically, extrahepatic and intrahepatic causes are described. In 35-41% of extrahepatic cases, biliary atresia is the cause. Intrahepatic causes are bacterial or viral neonatal infections (bacterial sepsis, hepatitis viruses, Coxsackie virus, varicella-zoster virus, Echovirus), TORCH syndrome, metabolic diseases (galactosemia being responsible for 37% of cases), genetic disorders such as alpha-1 antitrypsin deficit, cystic fibrosis, Alagille syndrome, progressive familial intrahepatic cholestasis, prematurity and intrauterine growth restriction (IUGR) [3,4].

In preterm and SGA babies, the etiology of the cholestasis is multifactorial, involving factors such as immaturity of the bile ducts and the excretory system (bile flow is reduced and hepatocytic transporters are immature), lack of enteral nutrition (the secretion of intestinal hormones is decreased and the enterohepatic circulation is altered), prolonged parenteral nutrition (closely related to the duration of its administration), sepsis, necrotizing enterocolitis (NEC), perinatal hypoxia and hemodynamic instability. The male gender is more frequently affected [5-8].

Intrauterine growth restriction is an independent risk factor for neonatal cholestasis. Intrauterine liver hypoperfusion in IUGR fetuses alters the liver's metabolic function. It lowers the digestive tolerance, and the histopathological changes in the liver are more severe in IUGR preterm infants compared to term newborn infants [8].

Another important risk factor for neonatal cholestasis is parenteral nutrition. Preterm and SGA newborn infants depend on parenteral nutrition for varying lengths of time. 18-67% of preterm infants who require total parenteral nutrition (TPN) for more than 14 days will develop neonatal cholestasis. Many factors favor parenteral nutrition-associated cholestasis: prematurity, neonatal sepsis, enteral nutrition delay, and the composition of the fluids used in parenteral nutrition. Soy-based lipid emulsions, which have an increased content of unsaturated fats and high levels of phytosterols, predispose to liver damage.

In this paper, we present the case of a preterm SGA baby with severe neonatal cholestasis, reviewing at the same time the risk factors associated with cholestasis in preterm SGA babies.

**CASE REPORT**

We present the case of a male newborn infant, 30 weeks gestational age, with birth weight 800 g, born by emergency cesarean section (placental infarction, meconium-stained amniotic fluid); Apgar scores were 6 at 1 minute, 8 at 5 minutes, requiring continuous positive airway pressure ventilation in the delivery room. His mother was 26 years old, gravida I, para I, and had pregnancy-induced hypertension treated with α-methyldopa (Dopexyl).

The infant was admitted to the neonatal intensive care unit (NICU) for respiratory distress syndrome and hypoglycemia. He required respiratory support (nasal CPAP, 30% oxygen) for 48 hours. Caffeine administration was initiated. Parenteral nutrition was provided: 4 mg/kg/min dextrose, 1 g/kg/day, amino acids, vitamins, and minerals. Upon admission, a blood culture was made, and antibiotic prophylaxis (ampicillin and gentamicin) was started.

Days 2-3 of age: minimal enteral nutrition was started (liquid milk formula – PreNAN Stage 1), but the newborn showed bloody gastric residuals with fresh blood, abdominal distension, bilious vomiting, and absence of meconium transit. A rectal tube was inserted, and a small amount of meconium was removed; it was adherent and whitish, with the appearance of silicon molds. Enteral feeding was stopped. Stool appearance raised the suspicion for cystic fibrosis or NEC. Abdominal ultrasound revealed thickened intestinal walls, pneumatocoele, diminished intestinal motility, and peritoneal fluid. Total parenteral nutrition was continued by adding lipids (1 g/kg SMOF lipid) and increasing protein supply up to 3.5 g/kg/day and lipids up to 2 g/kg/day. Enteral feeding was delayed for several days.

On the second day of life, jaundice was noted, initially with unconjugated bilirubin, but with progressive increase of the conjugated fraction of bilirubin, so that at 7 days postnatally, it reached 3.08 mg/dl while TSB level was 7.44 mg/dl. At this stage, the parenteral supply of lipids was stopped. Liver enzymes increased after the first 14 days of life (aspartate aminotransferase – AST, GGT, ALP) and triglycerides (Table 1). Lab work also demonstrated leukopenia and thrombocytopenia (Table 2).

Concurrently, on days 2-3 of life, the head ultrasound revealed a venous cerebral infarction located...
TABLE 1. Bilirubin and liver enzymes dynamics

<table>
<thead>
<tr>
<th>DOL</th>
<th>Direct bilirubin mg/dL</th>
<th>Total bilirubin mg/dL</th>
<th>Alanine amino transferase (ALT) UI/L</th>
<th>Aspartate amino transferase (AST) UI/L</th>
<th>Gamma glutamyl transferase (GGT) U/L</th>
<th>Alkaline phosphatase (ALP) U/L</th>
<th>Lactic dehydrogenase (LDH) U/L</th>
<th>Creatine kinase U/L</th>
<th>Triglycerides mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.67</td>
<td>6.02</td>
<td>11</td>
<td>69</td>
<td>150</td>
<td>92</td>
<td>875</td>
<td>1050</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.82</td>
<td>10.15</td>
<td>9</td>
<td>55</td>
<td>98</td>
<td>101</td>
<td>800</td>
<td>980</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>3.08</td>
<td>7.40</td>
<td>20</td>
<td>110</td>
<td>150</td>
<td>180</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>1.89</td>
<td>6.15</td>
<td>29</td>
<td>138</td>
<td>164</td>
<td>254</td>
<td>480</td>
<td>560</td>
<td>364</td>
</tr>
<tr>
<td>21</td>
<td>3.9</td>
<td>6.5</td>
<td>46</td>
<td>136</td>
<td>176</td>
<td>297</td>
<td></td>
<td></td>
<td>337</td>
</tr>
<tr>
<td>30</td>
<td>1.9</td>
<td>7.1</td>
<td>67</td>
<td>247</td>
<td>188</td>
<td>332</td>
<td></td>
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<tr>
<td>40</td>
<td>2.4</td>
<td>4.9</td>
<td>39</td>
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<td>180</td>
</tr>
<tr>
<td>50</td>
<td>1.29</td>
<td>2.7</td>
<td>30</td>
<td>77</td>
<td>287</td>
<td>125</td>
<td>178</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>1.01</td>
<td>1.5</td>
<td>48</td>
<td>70</td>
<td>309</td>
<td>974</td>
<td></td>
<td></td>
<td>125</td>
</tr>
</tbody>
</table>

Legend: DOL – day of life

TABLE 2. Blood count and coagulation determinations

<table>
<thead>
<tr>
<th>DOL</th>
<th>WBC</th>
<th>Hemoglobin (g/dL)</th>
<th>Hematocrit (%)</th>
<th>Platelets</th>
<th>Prothrombin time (PT) (seconds)</th>
<th>aPTT sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5480</td>
<td>14.9</td>
<td>49</td>
<td>93.000</td>
<td>93.000</td>
<td>14.9</td>
</tr>
<tr>
<td>2</td>
<td>5020</td>
<td>19.0</td>
<td>69</td>
<td>76.000</td>
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<td>69.0</td>
</tr>
<tr>
<td>3</td>
<td>4860</td>
<td>17.0</td>
<td>55</td>
<td>94.000</td>
<td>94.000</td>
<td>55.0</td>
</tr>
<tr>
<td>4</td>
<td>5360</td>
<td>14.0</td>
<td>43</td>
<td>47.000</td>
<td>47.000</td>
<td>43.0</td>
</tr>
<tr>
<td>5</td>
<td>5240</td>
<td>13.0</td>
<td>66</td>
<td>100.000</td>
<td>100.000</td>
<td>66.0</td>
</tr>
</tbody>
</table>

Legend: DOL – day of life

TABLE 3. Differential diagnosis

<table>
<thead>
<tr>
<th>Congenital sepsis</th>
<th>Blood culture</th>
<th>CRP (mg/dL)</th>
<th>Procalcitonin (ng/dL)</th>
<th>Negative 0,5/0,22 - Normal values 0,46/0,31 - Normal values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torch Syndrome</td>
<td>IgM CMV</td>
<td>IgM /IgA anti toxoplasma</td>
<td>Maternal serology for CMV, toxoplasmosis</td>
<td>Negative Negative Negative</td>
</tr>
<tr>
<td>Neonatal Hepatitis</td>
<td>Maternal serology for B and C hepatitis</td>
<td>ALT (UI/L)</td>
<td>AST (UI/L)</td>
<td>Negative 11/9/20 - Normal values 61/55/110 - Normal values</td>
</tr>
<tr>
<td>Biliary atresia</td>
<td>Abdominal ultrasound</td>
<td>Normal biliary tree and liver parenchyma</td>
<td>Normal gallbladder image</td>
<td></td>
</tr>
<tr>
<td>Cystic Fibrosis</td>
<td>Delta F508</td>
<td>Immunoreactive trypsinogen (ng/L)</td>
<td>The sweat test at 3month old</td>
<td>Negative 293 - Normal values Negative</td>
</tr>
<tr>
<td>Metabolic screening</td>
<td>Galactosemia, tyrosinemia, hypothyroidism, cystic fibrosis</td>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha 1 Antitrypsin</td>
<td>Normal values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alagille Syndrome</td>
<td>No facial dysmorphism</td>
<td>No congenital cardiac malformations</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Anterolateral to the frontal horn of the right lateral ventricle, evolving toward a porencephalic cyst adjacent to the right lateral ventricle. Bilateral leukomalacia was also noted.

Differential diagnosis (Table 3) included early neonatal sepsis, congenital infections (TORCH syndrome, neonatal hepatitis), biliary atresia, inborn errors of metabolism (galactosemia, tyrosinemia), genetic disorders (alpha-1 antitrypsin deficiency, cystic fibrosis, Alagille syndrome), congenital hypothyroidism.

The final diagnosis was SGA preterm infant, perinatal asphyxia, NEC, neonatal cholestasis of multifactorial etiology, cerebral infarction, and periventricular leukomalacia.

Treatment included: TPN through a central venous catheter that was kept in place for 28 days (4 mg/kg/min dextrose, 3.5 g/kg/day proteins, 200 mg/
kg/day arginine; 2 g/kg/day lipids, for 7 days; electrolytes, vitamins, calcium, and phosphorus); antibiotics (ampicillin and gentamycin then meropenem, vancomycin, metronidazole, fluconazole); blood products were also administered (platelet transfusion; fresh frozen plasma; packed red blood cells – 2 times). Enteral feeding suspended on day 3 of life was resumed with the mother’s milk at 12 days of age. The level of direct bilirubin warranted treatment with ursodeoxycholic acid orally. At 3 weeks after starting ursodeoxycholic acid, the conjugated bilirubin level was 1.29 mg/dl, and AST and ALT returned to normal values. GGT and ALP levels remained very high. Throughout this time, ammonia and coagulation tests remained within normal ranges.

DISCUSSION

Neonatal cholestasis in extremely low birth weight (ELBW) and SGA preterm infants is a multifactorial, often life-threatening disease. Its incidence in these babies is much higher than in term infants. In a 19-year retrospective study, Carneiro and colleagues found that the incidence of cholestasis in patients admitted to the NICU was 1% higher than in the general population. In preterm infants with gestational age less than 28 weeks, the incidence was 100-200 times higher than in term newborn infants due to the immaturity of the biliary tract [5].

In our case, the IUGR was the initial risk factor. Cholestasis is one of the complications described in IUGR newborn infants, with an increased mortality rate. Liver hypoperfusion in IUGR fetuses alters the metabolism of biliary acids, increasing the risk of parenteral nutrition-associated cholestasis. Baserga and colleagues showed that IUGR mice fetuses have low levels of ATP, and changes in the expression of genes that code for some liver enzymes and biliary acids metabolism are altered. These fetuses develop portal and sinusoidal fibrosis secondary to parenteral nutrition [9]. Moreover, perinatal asphyxia is an additional risk factor for neonatal cholestasis. Herzog and colleagues found an increased incidence of cholestasis in SGA neonates with perinatal asphyxia (33%) compared to AGA neonates with perinatal asphyxia (8.5%). They conclude that IUGR, male gender, and gestational age below 35 weeks increase the risk for cholestasis in preterm infants with perinatal asphyxia [10]. Our case was a male SGA preterm infant with Apgar Score 6 at 1 minute who required resuscitation in the delivery room, with severe complications in the NICU: intracranial hemorrhage and necrotizing enterocolitis.

The role of parenteral nutrition in triggering neonatal cholestasis is mentioned in numerous studies, as early as the 70s-80s, being strongly correlated to the duration of TPN. In AGA preterm newborns, cholestasis appears after 14 days of TPN, whereas in IUGR preterm infants, the onset is earlier, noted after only 7 days of TPN, and it lasts longer [11-12]. Severe forms, with conjugated bilirubin levels increasing above 4 mg/dl that last for more than one month and associated with increased liver enzymes, are more frequent in SGA preterm infants compared to those appropriate for gestational age (AGA) [13]. However, Costa and colleagues showed that SGA preterm infants do not have an increased risk for TPN-associated cholestasis [14].

TPN-associated cholestasis is correlated to the composition of TPN solutions. Soy-based lipid emulsions, with an increased content of ω-6 and phytosterols, increase the risk of cholestasis. These stimulate the production of pro-inflammatory prostaglandins (PgE2) and thromboxane A2, which induce immune response and liver damage. In animal studies, phytosterol inhibits the proteins involved in bilirubin and bile acid excretion [15]. Instead, mixed vegetable and fish oil-derived lipid emulsions have lower phytosterol content. They are rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), with immunomodulatory and anti-inflammatory effects [16-19]. At the same time, the amounts of amino acids used in TPN and the composition of amino acid solutions increased the risk of cholestasis. The increased amounts of amino acids used in TPN affect liver function through direct damage of hepatocytes, thus leading to cholestasis. Regarding the composition of amino acid solutions, taurine has a protective effect against cholestasis, while methionine, tryptophan, and phenylalanine damage the membrane of hepatocytes [19]. In our case, total amino acids intake was 3.5 g/kg/day, and lipid intake was 2 g/kg using SMOF lipid emulsion containing triglycerides, soybean oil, olive oil, and fish oil.

Delay of enteral nutrition is a factor that closely connects with the duration of parenteral nutrition. Decreased hormones, digestive enzymes, and decreased enterohepatic circulation lower the bile flow and favor cholestasis. Last but not least, the absence of trophic enteral nutrition causes damage to the intestinal wall, which becomes permeable to bacteria and their toxins. Gram-negative germs release endotoxins that induce inflammatory response at the Kupffer cells level. The duration of antibacterial therapy and the use of antifungals contribute to all this [8]. In our case, although parenteral lipid supply was only 1 g/kg, and the emulsion used was based on fish oil (SMOF lipid), the early NEC onset and the lack of enteral nutrition for many days constituted factors that favored neonatal cholestasis. The use of antibiotic and antifungal therapy (ampicillin, gentamicin, meropenem, vancomycin, and fluconazole) for many days added to these.
The initial absence of intestinal transit and the persistence for several days of stools with silicone mold appearance raised suspicion for cystic fibrosis. This diagnosis could not be confirmed: delta F508 mutation was negative, and immunoreactive trypsin was within normal limits. The screening for inborn errors of metabolism was carried out late (not included in the national screening program), and its results were difficult to interpret. So, at 3 months of age, the sweat test was performed, finally removing this suspicion.

The clinical and paraclinical evolution was slowly favorable once ursodeoxycholic acid was introduced. After 3 weeks from the initiation of treatment, conjugated bilirubin dropped dramatically, and transaminases returned to normal values. However, GGT and ALP remained high for a long time, returning within normal limits at 90 postnatal days. It is well known that GGT has higher values in premature newborns than in healthy-term newborns. GGT is the most reliable marker of inflammation of the bile ducts [5]. However, Teng and colleagues showed that in preterm infants below 30 weeks of gestational age, although cholestasis is associated with numerous complications and high mortality, there is no long-term liver damage in survivors [20].

CONCLUSIONS

Cholestasis is a complex diagnostic with multifactorial etiology in SGA preterm infants requiring additional care to prevent life-threatening complications. Early recognition and diagnosis are life-saving, so the treatment should be initiated as early as possible.

Patient consent:
Informed consent was obtained for the use of data from medical records.

Conflict of interest:
The authors declare no conflict of interest.

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