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case report

Calcium Oxalate-Phosphate Gallstones, A Unique Chemical Type of Gallstone

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Cholelithiasis is rare in infants. When present in infants, gallstones are usually composed of bilirubin and cholesterol. We report a premature neonate who developed calcium oxalate-phosphate gallstones, detected at four months of age. Calcium oxalate-phosphate gallstones are uncommon in both infants and adults, and the pathogenesis is unknown.

Additional Keyphrases: *cholelithiasis · pediatric chemistry · newborns · respiratory distress syndrome · parenteral nutrition · furosemide*

Cholelithiasis is a rare disease in infants. When gallstones are present in this age group, they are usually composed of bilirubin (resulting from hemolysis) or cholesterol (caused by abnormalities of the biliary tract or the enterohepatic circulation of bile acids). A recent report (*1*) described gallstones that occurred in three infants with bronchopulmonary dysplasia who were treated with furosemide and parenteral nutrition. We encountered a similar patient whose gallstones were composed of calcium oxalate-phosphate. We found no report in the literature of gallstones of such composition in either infants or adults, and this unusual circumstance is the subject of our report.

Case Report

This 1190-g female infant was born at home to a 21-year-old white woman (gravida 2, para 1, spontaneous abortion 1). The newborn was promptly brought to the Infant Special Care Nursery because of respiratory distress. Estimated gestational age was 29 weeks. By clinical and roentgenographic criteria, the infant had respiratory distress syndrome, which was treated with assisted ventilation. A patent ductus arteriosus was ligated on the second postnatal day. The patient required assisted ventilation during the succeeding month, as well as total parenteral nutrition, which included Intralipid.

Despite the development of bronchopulmonary dysplasia, the patient tolerated extubation after one month and discontinuation of parenteral nutrition. Her lung disease was treated with furosemide and theophylline. Repeated episodes of respiratory insufficiency during the remaining five months of life demanded intervals of assisted ventilation, and the addition of spironolactone and digoxin; altogether, she required 75 days of assisted ventilation. A total of 108 mL of packed erythrocytes was administered for iatrogenic blood loss during her course.

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Candida albicans arthritis of the knee, detected at two months of age, was treated with amphotericin B and fluorocytosine for the next six weeks. At three months of age, the patient was treated with acyclovir (60 mg/kg per day) for 10 days after cytomegalovirus was isolated from the trachea and urine, with no discernible benefit.

The patient died of respiratory insufficiency at six months of age.

Abdominal calcifications in the right upper quadrant were first noted at four months of age (Figure 1). Bilateral decubitus radiographs showed that the calcifications changed in their relative locations with positional changes, and projected anteriorly, suggesting gallstones free in the gallbladder; this was confirmed sonographically. Other roentgenographic findings included severe bronchopulmonary dysplasia and marked, generalized osteopenia.

At autopsy, severe bronchopulmonary dysplasia (*2*) with right ventricular hypertrophy was seen. There was hypertrophy of hepatocytes, without cholestasis or cholangitis. The gallbladder was of normal size, but contained three gallstones stained black, each weighing about 70 mg. There was no thickening of the gallbladder wall or evidence of acute or chronic inflammation. There was no obstruction, infection, or congenital anomaly of the biliary tract. The pancreas and ileum were normal, but there was mild hemorrhagic gastritis and an area of focal chronic inflammation in the sigmoid colon. There was no evidence of primary systemic oxalosis. By chemical analysis, the gallstones were mostly composed of calcium oxalate monohydrate [83% $CaC_2O_4 \cdot H_2O$, 9% $Ca_{10}(PO_4)_6(OH)_2$, and 8% protein], without bile pigments or cholesterol. Analysis for furosemide was not performed.

Methods

Analysis of the gallstones' composition was performed by three procedures. The first, the oxalic oxidase procedure, involves the use of oxalate oxidase (oxalate:oxygen oxidoreductase, EC 1.2.3.4) (*3*). This enzyme, which occurs in moss and barley roots, produces 2 mol of carbon dioxide and 1 mol of hydrogen peroxide from 1 mol of oxalic acid. The hydrogen peroxide generated from oxalate breakdown is quantitated by measuring the absorbance of chromophore formed by horseradish peroxidase (EC 1.11.1.7) catalyzed oxidation of a chromogen. Interfering substances, particularly divalent metals and ascorbic acid, are removed by ion-exchange chromatography and charcoal oxidation. In the second method, diphenylamine is added to an equivalent amount of the calculus, followed by two drops of phosphoric acid and heating at 245 °C for 1 min. After cooling the solution, an equal volume of 95% ethanol is added, giving the characteristic blue color. Finally, we also used infrared analysis, in which a potassium bromide window was made by grinding the calculi with potassium bromide (1 mg of sample to 100 mg of potassium bromide). The infrared scan was positive for oxalate. The

Fig. 1. Radiograph of patient's abdomen at four months of age; gallstones are indicated by *arrows*

calculi appeared black because of bile staining from bile in the gallbladder.

Discussion

The unique feature of this case is the presence of calcium oxalate-phosphate gallstones. The presence of appreciable oxalate in gallstones has not been described previously in studies of the composition of calcium-containing gallstones (*4*, *5*).

The pathogenesis of oxalate-containing gallstones is unknown. Oxalate is not present in bile in appreciable concentrations (*6*). The patient did not receive exogenous oxalate, so the source of the oxalate in the gallstones is probably fungal, bacterial, or tissue synthesis of oxalate or a precursor of it. There are old reports showing that oxalate may be formed by fungi (*7*) and that glyoxylate, its immediate precursor, is formed by bacteria (*8*) but demonstration of oxalate formation by microorganisms in vivo has not been shown. A slight but definite normal production of endogenous oxalate occurs, dependent on diet (*6*). Such endogenous oxalate is increased in patients with hyperoxaluria after jejunoileal bypass surgery for obesity (*9*).

The previously reported cases of infants with cholelithiasis had similar clinical features: prematurity, respiratory distress, and the development of bronchopulmonary dysplasia with prolonged total parenteral nutrition and furosemide administration. Unfortunately, in those cases gallstone analysis was not performed, or was limited and demonstrated minor amounts of bilirubin (*1*). Furthermore, our patient had none of the conditions usually associated with gallstone formation in young children.

Patients with hemolytic anemia develop bilirubin pigment gallstones; this association is thought to be related to increased secretion of unconjugated bilirubin into bile. Cholelithiasis may also complicate biliary tract abnormalities such as choledochal cysts, as well as anomalies of the cystic duct and stenosis of the common bile duct. Presumably, stasis of bile flow permits bacterial proliferation, with deconjugation of bilirubin and hydrolysis of lysolecithin. Bilirubin deconjugation during transit in the biliary tract, mediated by bacterial or lysosomal glucuronidase, by serving as a nucleus for cholesterol crystallization, may cause calcium bilirubinate gallstones and may contribute to cholesterol gallstone formation. Hydrolysis of lecithin may also contribute to cholesterol gallstone formation because the fatty acid formed is absorbed by the gallbladder, decreasing the cholesterol solubilizing capacity of bile. Ileal disease, resection, or bypass are also associated with increased formation of gallstones. The interruption of the enterohepatic cycle of bile acids leads to shrinkage of the bile acid pool, producing a relative excess of cholesterol in bile (*10*).

Our patient received hyperalimentation, which has been suggested as another possible etiological factor in cholelithiasis in neonates (*1*). The proposed mechanism is decreased bile acid clearance due to intrahepatic cholestasis, induced by the hyperalimentation mixture.

Finally, another factor that may have been important in the pathogenesis of calcium oxalate gallstones in our patient is prolonged treatment with various medications, of which furosemide is perhaps the most suspect. The prolonged treatment with fuorsemide resulted in hyponatremia, hypokalemic metabolic alkalosis, and osteomalacia with hypercalciuria. Furosemide may have enhanced calcium excretion into the bile (*1*, *11*), which would have promoted precipitation of calcium-containing calculi.

In cases similar to this one, the calculi should receive a full chemical analysis.

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