Fat Malabsorption due to Bile Acid Synthesis Defect

Firas Rinawi MD[1], Theodore C. Iancu MD[5], Corina Hartman MD[1,3], Hofit Cohen MD[4], Havatzelet Yarden-Bilavsky MD[2,3], Michal Rozenfeld Bar Lev MD[1] and Raanan Shamir MD[1,3]

[1]Institute of Gastroenterology, Nutrition and Liver Diseases, and [2]Department of Pediatrics A, Schneider Children's Medical Center of Israel, Petah Tikva, Israel
[3]Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel
[4]Strassburger Lipid Center, Sheba Medical Center, Tel Hashomer, Israel
[5]Milman-David Biomedical Research Unit, Haifa, Israel

KEY WORDS: fat malabsorption, steatorrhea, cholestatic liver disease, bile acids synthesis, hypocholesterolemia

IMAJ 2015; 17: 190–192

Primary bile acids (cholic and chenodeoxycholic acid) are sterol compounds synthesized from cholesterol in the liver. Primary bile acids (BA) are essential for the absorption of fat and fat-soluble vitamins, being responsible for fat emulsion and micelles formation, which facilitate the action of pancreatic lipase and enhance fat absorption. Defects in BA synthesis lead to reduced production of primary bile salts and usually present as neonatal cholestasis, fat malabsorption and steatorrhea [1].

Impaired fat digestion or absorption and steatorrhea have multiple etiologies (pancreatic insufficiency, cholestatic liver disease, diffuse mucosal/enterocyte disease, defects in synthesis, secretion of chylomicrons/lipoproteins, and disorders of the lymphatic system). Untreated, fat malabsorption may result in malnutrition, growth failure, and deficiencies in fat-soluble vitamins A, E, D and K, with resultant neurological deficits, rickets/osteomalacia, coagulopathy, visual impairment and skin changes. Any defect in the process of fat absorption can cause major clinical symptoms and biochemical changes, which may even be life-threatening in extreme conditions.

PATIENT DESCRIPTION

A 2.5 year old male child was admitted due to failure to thrive, chronic diarrhea and fat-soluble vitamin deficiencies. The child was the youngest of four children of healthy, consanguineous Ashkenazi Jewish parents. He was born at term without perinatal or postnatal complications and had no remarkable medical history during his first year of life. He started having diarrhea and failed to gain weight appropriately from the age of 1 year. He was hospitalized at age 2 with fever and macrohematuria with elevated internal normalized ratio (INR) (more than 10 times the upper limit of normal). Apart from these findings, the child also had microcytic anemia (hemoglobin 7.6 g/dl) and hypoalbuminemia (3.2 g/dl). Other laboratory tests were normal, including thrombocyte count, bilirubin and liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT)]. Celiac serology was negative, sweat test and stool elastase were normal, as was upper endoscopy including intestinal histology. Abdominal sonography demonstrated nephrocalcinosis. The INR normalized with vitamin K treatment and the macrohematuria resolved. The child was discharged with fat-soluble vitamins (ADEKs) and vitamin E supplementation. During ambulatory follow-up, the hypoalbuminemia and INR normalized, but slight elevation of liver enzymes without hyperbilirubinemia was observed on repeated tests (ALT 50 IU/ml, AST 50 IU/ml, normal GGT 12 IU/ml). In addition, deficiencies of vitamin A (11 µg/dl, normal range 30–120 µg/dl), vitamin D (16 ng/ml, normal 20–100 ng/ml), and vitamin E (0.0 µg/ml/undetectable, normal 5–20 µg/ml) were detected before the supplementation was begun.

The child was admitted again for further investigation and follow-up. His weight and height were on the 10th percentiles, and physical/neurological examinations were normal at admission. Repeated laboratory tests revealed elevated liver enzymes (ALT up to 120 IU/ml, AST up to 75 IU/ml); elevated INR (2.1); normal bilirubin, albumin and GGT; low total cholesterol levels (61–91 mg/dl), low levels of low density lipoprotein (LDL) cholesterol (23–36 mg/dl) (LDL direct); and normal triglyceride levels (42–74 mg/dl).

A repeat abdominal sonography demonstrated hepatomegaly and periportal fibrosis. Liver autoimmune workup, alpha-1-antitrypsin blood levels and phenotype, as well as infection workup were normal. Serum total BA and essential fatty acids were within the normal range of age. Apolipoprotein levels were decreased: ApoA1 was 79 mg/dl (normal range 100–200 mg/dl), ApoB was 35 mg/dl (normal 40–125 mg/dl). Lipoproteins were isolated from plasma by ultracentrifugation: LDL, high density lipoprotein (HDL) and very low density lipoprotein (VDLD) fractions revealed a normal pattern. No chylomicrons in a postprandial sample were detected. These results, in combination with hypocholesterolemia and normal serum triglycerides, were suggestive of chylomicron retention disease. However, repeated upper gastrointestinal endoscopy, including intestinal biopsies with light and electron microscopy, demonstrated normal structure of enterocytes (ruling out villous atrophy/microvillus inclusion disease, chylomicron retention disease or intestinal lymphangiectasia) [Figure 1A]. Furthermore, liver biopsies for light and electron microscopy showed no evidence of liver steatosis, a finding inconsistent with the diagnosis of chylomicron retention disease. The liver biopsy, however, demonstrated active liver disease with bridging fibrosis and an ongo-

ing destructive process with severe mitochondrial alterations, prominent increase in peroxysomes, abnormal shape of the nuclei, and occasional detachment of the membrane of the hepatocyte [Figure 1B].

Due to the suspicion of a defect in BA synthesis (liver damage and fat malabsorption with normal GGT and serum bile acids levels), we performed analysis of urinary BA metabolites by electrospray ionization tandem mass spectrometry. All the major fractions of urinary BA metabolites (3β,7α-dihydroxy-5-cholenoic acid, 3β,7α,12α-trihydroxy-5-cholenoic acid and their glycine conjugates) were sulphated. This finding is typical for 3β-hydroxy-Δ5-C27-steroid dehydrogenase deficiency. The diagnosis was confirmed by HSD3B7 gene sequencing and the patient was found to be homozygous for C.1031A>G; p.Tyr344Cys mutation.

There are two major pathways of bile acid synthesis in the liver: neutral and acidic [1]. In the case of 3β-hydroxy-Δ5-C27-steroid dehydrogenase (3β dehydrogenase) deficiency there is a total deficiency in synthesis of both cholic (CA) and chenodeoxycholic acid (CDCA) via the neutral pathway. However, some CDCA is produced via the acidic pathway. CA therapy is the treatment of choice for this enzymatic defect, as negative feedback secondary to CA administration will prevent upstream accumulation to toxic metabolites and further liver damage. However, this drug has not yet been approved by the Food and Drug Administration (FDA). Treatment with CDCA is also acceptable [1], and we started this treatment after diagnosis, beginning with a high dose of 15 mg/kg per day for 2 months followed by 10 mg/kg/day maintenance, both as a twice-daily regimen. A remarkable clinical improvement was noted within the first 6 months of treatment, including weight gain, resolution of diarrhea and normalization of serum lipids and fat-soluble vitamin levels. Furthermore, fat-soluble vitamin levels remained normal even after discontinuing supplementation with ADEK and vitamin E. Repeated liver sonography demonstrated a regression in periportal fibrosis and hepatomegaly. Side effects were not reported. Despite clinical and sonographic improvement, 6 months after the initiation of CDCA treatment, liver enzymes (AST and ALT) remained high.

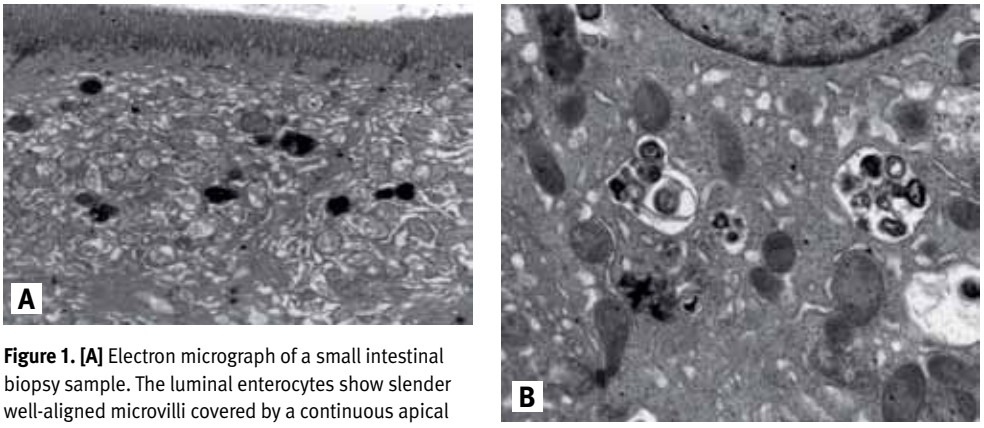

Figure 1. [A] Electron micrograph of a small intestinal biopsy sample. The luminal enterocytes show slender well-aligned microvilli covered by a continuous apical glycocalyx. There are no abnormal inclusions or other pathological findings in the lamina propria. These findings exclude the diagnosis of microvillus inclusion disease **[B]** Electron micrograph of a hepatocyte with parenchymal alterations. Numerous single-membrane-limited bodies are seen. They contain whorls consistent with lysosomal residual bodies; such lysosomal bodies are found in various metabolic abnormalities, with or without cholestasis (CBN = cytoplasmic biliary necrosis) and even without jaundice. Mitochondria show various alterations, including cristolysis and abnormal size and shape

Repeated analysis of urinary BA metabolites demonstrated markedly reduced excretion of di- and tri-hydroxy5-cholenoic acids compared to pretreatment levels and showed some saturated bile acids including glycodihydroxycholanoates, glycotrihydroxycholanoates, and taurotrihydroxycholanoates. However, the excretion of unsaturated bile acids was still greater than of saturated bile acids, indicating that CDCA treatment was not able to fully block the production of upstream toxic metabolites. Therefore, about 1 year after the diagnosis, the child was started on CA, as part of a study undertaken at Cincinnati Children's Medical Center [2]. At present, 12 months on CA treatment at a dose of 15 mg/kg per day, the child has no clinical symptoms or laboratory findings suggestive of fat malabsorption, and repeated analysis of urinary BA metabolites has demonstrated markedly reduced excretion of unsaturated bile acids.

COMMENT

Bile acids are synthesized in the liver from cholesterol by a complex series of chemical reactions catalyzed by 17 different hepatic enzymes located in different subcellular fractions [3]. There are multiple pathways in bile acid synthesis, but irrespective of the pathway by which unconjugated cholic and chenodeoxycholic acids are formed, the final step leads to the formation of the glycine and taurine conjugates. These bile acids account for 95% of the BA secreted in bile and are responsible for driving bile flow. At the genetic level, nine inborn errors in the bile acid biosynthetic pathways have been described to date: cerebrotendinous xanthomatosis, 3β-hydroxy-Δ5-C27-steroid dehydrogenase/isomerase deficiency, Δ4-3-oxosteroid 5β-reductase (5β-reductase) deficiency, oxysterol 7α-hydroxylase deficiency, cholesterol 7α-hydroxylase deficiency, as well as the peroxisomally located defects 2-methylacyl-CoA racemase (AMCAR) deficiency, D-bifunctional protein deficiency, and sterol carrier protein X (SCPx) deficiency. Although inborn errors in BA synthesis involving defective synthesis of cholic and chenodeoxycholic acids usually present as progressive cholestatic liver disease [3], cholestasis may not be the primary manifestation of these disorders. Liver disease secondary to BA synthesis defects often presents in early childhood with jaundice, acholic stools, hepatomegaly and fat-soluble vitamin

deficiencies. Serum aminotransferases and conjugated bilirubin are usually elevated and, typically, GGT activity is normal. The pathophysiology of the clinical manifestations in 3β-dehydrogenase deficiency and other BA synthesis defects are related to several main factors: impaired bile flow, low concentration of primary bile acids in the intestinal lumen, fat-soluble vitamin malabsorption, and liver injury secondary to BA hepatotoxic metabolite accumulation.

3β-hydroxy-Δ5-C27-steroid dehydrogenase (3β-dehydrogenase) deficiency, the most common BA synthesis defect, affects the conversion of 7α-hydroxycholesterol to 7α-hydroxy-4-cholesten-3-one. Although the clinical presentation is heterogeneous, most patients present with neonatal cholestasis. Liver histology shows generalized hepatitis, giant cell transformation, and cholestasis [4]. The simplest and definitive test for the diagnosis of BA synthesis disorders is analysis of urinary and plasma cholanoids (bile acid and bile alcohol) profile by electrospray ionization tandem mass spectrometry. In the case of 3β-dehydrogenase deficiency there are two additional tests for the diagnosis: measurement of enzymatic activity in fibroblasts, and sequencing of the HSD3B7 gene which encodes the defective enzyme. 3β-dehydrogenase deficiency responds extremely well to BA replacement therapy. BA replacement treatment results in normalization of liver enzymes and liver function tests, morphological improvement in liver biopsy, correction of fat-soluble vitamin malabsorption, weight gain and growth improvement. This has been achieved by administration of cholic and/or chenodeoxycholic acid, with or without the addition of ursodeoxycholic acid (UDCA). Treatment can be monitored by suppression of urinary excretion of unsaturated bile acids [1]. The main treatment goals are: first, to down-regulate the activity of enzymes proximal to the defect and thus decrease the production of non-functional hepatotoxic metabolites; and second, to increase the primary bile acid pool, improve cholestasis, and facilitate fat and fat-soluble vitamin absorption. Treatment with UDCA alone is not effective in suppressing the production of hepatotoxic metabolites and hepatic damage progress. Combination of UDCA with CA or CDCA is controversial due to the risk that UCDA may interfere with the absorption of CA or CDCA in the terminal ileum leading to a decrease in primary bile acids pool and worsening fat malabsorption.

In our case, treatment with CDCA, despite improving fat and fat-soluble vitamin absorption, failed to achieve the goal of suppressing production of hepatotoxic bile acid metabolites. This can be explained by the fact that 3β-dehydrogenase deficiency blocks the production of CA via the neutral pathway; therefore, to induce effective suppression of hepatotoxic metabolite production, replacement therapy with CA rather than CDCA is needed. The persistent elevation of the patient's liver enzymes during the 6 months of treatment with CDCA may be explained by the longer duration needed for hepatic damage regression and does not necessarily indicate insufficient treatment. Even during treatment with CA, normalization of liver enzymes may take months to years. Treatment with CA in 3β-dehydrogenase deficiency is considered to be safe, effective and lifesaving [2]. Delaying CA treatment carries the risk of disease progression to hepatic failure, severe malnutrition and neurological manifestations due to vitamin E deficiency [1,3].

Besides the two major pathways of bile acid synthesis (neutral and acidic), there are other compensatory pathways such as Yamasaki and 25-hydroxylation pathways that play an important role in bile acid synthesis in stress situations and in the neonatal period. For example, in cerebrotendinous xanthomatosis (CTX), a storage lipid disorder characterized by ineffective elimination of cholesterol and cholestanol deposition in extrahepatic tissues, there is no liver disease and CA production is adequate. In CTX there is a deficiency of mitochondrial sterol 27-hydroxylase, which is an essential enzyme in both acidic and neutral pathways. Production of CA and CDCA via both the acidic and the neutral pathways is therefore suppressed; however, CA synthesis by the 25-hydroxylation pathway explains the absence of liver disease and the adequate level of CA. Replacement therapy with CDCA is effective in CTX and may prevent neurological deterioration.

In summary, physicians should suspect BA synthesis defects in the context of fat malabsoprtion even in the absence of cholestasis, especially in the presence of normal serum bile acids. Furthermore, this case provides some unique observations: first, neurological examination, including deep tendon reflexes, was normal despite undetectable serum vitamin E. This observation is probably explained by the short duration of vitamin E deficiency. Second, despite severe fat-soluble vitamin deficiencies, plasma levels of essential fatty acids were in the normal range for age [5], suggesting that fat-soluble vitamin transport is more sensitive to BA synthesis defect than is fat absorption. Also interesting were the histological abnormalities observed on electron microscopy of the liver biopsy (especially the extensive mitochondrial alteration), which have not been described in the literature.

In conclusion, inborn errors of bile acid biosynthesis usually present as cholestasis but can also present as fat malabsorption. Early diagnosis of these disorders is essential, as effective treatment with BA replacement therapy is readily available and permanent neurological damage may ensue as a result of delayed diagnosis.

Correspondence
Dr. F. Rinawi
Institute of Gastroenterology, Nutrition and Liver Diseases, Schneider Children's Medical Center of Israel, Petah Tikva 49202, Israel
email: firasri@clalit.org.il

References

1. Clayton PT. Disorders of bile acid synthesis. *J Inherit Metab Dis* 2011; 34: 593-604.
2. Gonzales EM, Gerdhardt MA, Fabre MO, et al. Oral cholic acid for hereditary defects of primary bile acid synthesis: a safe and effective long-term therapy. *Gastroenterology* 2009; 137: 1310-20.
3. Setchell KD, Heubi JE, Shah S, et al. Genetic defects in bile acid conjugation cause fat-soluble vitamin deficiency. *Gastroenterology* 2013; 144: 945-55.
4. Clayton PT, Leonard JV, Lawson AM, et al. Familial giant cell hepatitis associated with synthesis of 3b, 7a-dihydroxy and 3b,7a,12a-trihydroxy-5-cholenoioc acids. *J Clin Invest* 1987; 79: 1031-8.
5. Glaser C, Demmelmair H, Sausenthaler S, Herbarth O, Heinrich J, Koletzko B. Fatty acid composition of serum glycerophospholipids in children. *J Pediatr* 2010; 157: 826-31.