Sitosterolemia Presenting With Severe Hypercholesterolemia and Intertriginous Xanthomas in a Breastfed Infant: Case Report and Brief Review

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Context: Sitosterolemia is an autosomal recessive disorder characterized by increased intestinal absorption of plant sterols. It is caused by mutations in genes encoding ATP-binding cassette, subfamily G5 (ABCG5) or G8 (ABCG8), and clinical features include elevated plant sterol levels, xanthomas, and accelerated atherosclerosis. Although it was originally reported in patients with normolipemic xanthomas, patients with sitosterolemia also hyperabsorb cholesterol, and serum cholesterol levels tend to be elevated.

Objective: We report an infant with sitosterolemia who presented with severe hypercholesterolemia and intertriginous xanthomas.

Case Report: A 15-month-old Korean girl presented with yellow dermal plaques over flexural areas including the wrist, neck, and gluteal folds, which were consistent with intertriginous xanthomas. The lesions were first noticed at 3 months of age when she was being exclusively breastfed. Her total cholesterol and low-density lipoprotein-cholesterol levels were 675 and 540 mg/dL, respectively. A low-fat/low-cholesterol diet and cholestyramine therapy were introduced. Unexpectedly, her serum cholesterol level decreased dramatically and normalized in 2 months. Cholestyramine was tapered off. The xanthomas also regressed and disappeared by 3 years of age. Gas chromatography-mass spectrometric analysis was performed with serum drawn at 3 years of age when her low-density lipoprotein-cholesterol was 118 mg/dL, which revealed striking elevation of her sitosterol level at 19.36 mg/dL. Direct sequencing for ABCG5 revealed compound heterozygous null mutations c.904+1G>A (p.Met302Asnfs*82) and c.1336C>T (p.Arg446*).

Conclusions: Our case suggests that sitosterolemia can present with severe hypercholesterolemia and intertriginous xanthomas. Sitosterolemia should be suspected when a patient with hypercholesterolemia shows unexpectedly good response to dietary modification or bile acid sequestrant therapy. (U Clin Endocrinol Metab 99: 1512–1518, 2014)
in patients with sitosterolemia, especially in children (6). In contrast to other forms, hypercholesterolemia in patients with sitosterolemia is unusually responsive to a low-cholesterol diet and/or bile acid sequestrants (1).

Xanthomatosis is rarely observed in infants, and when present, homozygous familial hypercholesterolemia (FH) or autosomal recessive hypercholesterolemia is most often suspected (7, 8). Intertriginous xanthomas are a very rare type of planar xanthomas and have been reported to be pathognomonic for homozygous FH (9). We report an infant presenting with intertriginous xanthomas and severe hypercholesterolemia, which were reversed by dietary modification and cholestyramine therapy. The child was finally diagnosed with sitosterolemia due to compound heterozygous mutations in \textit{ABCG5}.

**Clinical History**

A 15-month-old Korean girl presented with multiple yellow dermal plaques over the flexural areas. The lesions were first noticed by her parents at 3 months of age when she was being exclusively breastfed. Breast milk comprised one-third of her daily energy intake at her initial visit. Solid foods, mainly rice, meats, and vegetables, had been introduced at 6 months of age and were two-thirds of her daily energy intake. She was born to healthy nonconsanguineous parents, and there was no family history of dyslipidemia or premature cardiovascular disease.

Her height and weight were both between 25th–50th percentiles. Cutaneous examination showed multiple yellowish plaques on the wrist, neck, and gluteal folds, which were consistent with intertriginous xanthomas (Figure 1). There was no goiter or hepatomegaly. Total cholesterol and LDL-C were markedly elevated at 675 and 540 mg/dL, respectively. High-density lipoprotein-cholesterol and triglyceride levels were 46 and 51 mg/dL, respectively. The lipid profiles of her parents were all normal. Biopsy of the dermal plaques confirmed xanthoma with diffuse dermal foam cell proliferation (Supplemental Figure 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org).

A low-saturated-fat/low-cholesterol diet was introduced, and cholestyramine (0.24 g/kg/d) was started at 16 months of age. Unexpectedly, the cholesterol levels decreased dramatically, and normalized within 2 months (Figure 2). As the lipid profile was maintained in the low-normal range, cholestyramine was tapered gradually and discontinued at 25 months of age. The xanthomas also began to regress and disappeared by 3 years of age (Figure 1). Her development was normal, but her growth decelerated to the 10th–25th percentile of height and the 5th–10th percentile of weight at 54 months of age.

A plasma sample at 3 years of age, when her total cholesterol and LDL-C levels were 184 and 118 mg/dL, respectively, was kept frozen at −80°C until the analysis of plant sterols later. The gas chromatography-mass spectrometry (GC-MS) performed at 56 months of age revealed an extremely high plasma sitosterol level at 19.36 mg/dL (normal range, 0.3–1.7 mg/dL) (10). At this time, a diet with plant sterol restriction was introduced. Cholestyramine was restarted at 61 months of age and was replaced with ezetimibe at 64 months of age.

The patient’s 5-year-old sister was essentially asymptomatic, but her total cholesterol was 284 mg/dL, and LDL-C was 210 mg/dL. The total cholesterol and LDL-C decreased to 188 and 138 mg/dL, respectively, after 2 months of a low-saturated-fat/low-cholesterol diet. Her serum sitosterol, measured at 7 years of age after 6 weeks of a plant sterol-restricted diet, was 19.85 mg/dL.

**DNA Analysis**

Genomic DNA was extracted from peripheral blood leukocytes from the proband and the other family members. The protocol was approved by the Institutional Review Board of UT Southwestern Medical Center. Written informed consent was obtained from the parents. Because of increased prevalence of mutations in \textit{ABCG5} in sitosterolemia patients of Asian origin, we sequenced all exons and adjacent splice sites of \textit{ABCG5}. Primers used for genomic amplification and sequencing of \textit{ABCG5} were according to a previous report (1).

Sequencing revealed that the proband had compound heterozygous mutations c.904+1G>A (p.Met 302Asnfs*82) and c.1336C>T (p.Arg446*) in \textit{ABCG5}. Her sister also had the same compound heterozygous mutations,
and both parents were heterozygous carriers for each mutation (Figure 3).

Discussion

We confirmed the diagnosis of sitosterolemia in a breast-fed infant with severe hypercholesterolemia and intertriginous xanthomas. The lipid profiles and the presence of intertriginous xanthomas were highly suggestive of homozygous FH; however, the family history suggested an autosomal recessive condition, such as autosomal recessive hypercholesterolemia or sitosterolemia. Hyperlipidemia and xanthomas were reversed by a low-saturated-fat/low-cholesterol diet and cholestyramine therapy, whereas plant sterol levels were still very high after normalization of plasma cholesterol level.

Structurally, plant sterols have the same squalene ring nucleus as cholesterol, but they differ by the addition on the side chain of an ethyl or methyl group (sitosterol and campesterol, respectively) or the presence of a double bond (stigmasterol) (3). The dietary intake of plant sterols ranges from 150 to 450 mg/d (11). Although approximately 50% of dietary cholesterol is absorbed, only <5% of plant sterols are absorbed in normal individuals. Patients with sitosterolemia absorb 15 to 60% of ingested sitosterol (1, 3). Sitosterol is usually the most abundant plant sterol in the diet and proportionally the predominant form found in patients with sitosterolemia (11). Routine laboratory methods do not distinguish plant sterols from cholesterol, and a more accurate method such as GC-MS is required.

Sitosterolemia is caused by homozygous or compound heterozygous mutations in either ABCG5 or ABCG8 (1, 2). ABCG5 or ABCG8 is coexpressed almost exclusively in the intestine and liver, where they form heterodimers and actively pump sterols, especially plant sterols, from enterocytes into the gut lumen and from hepatocytes into the bile (12). Increased intestinal absorption and impaired biliary excretion of plant sterols lead to a 50- to 200-fold increase in their levels in sitosterolemia. Most Asian probands with sitosterolemia, including our case, have mutations in ABCG5, whereas most Caucasian probands have ABCG8 mutations (1, 6, 13). Heterozygotes are asymptomatic, and their plant sterol levels are slightly increased (3).

Based upon 80–100 cases reported in the world literature, the estimated prevalence of sitosterolemia is less than 1 in one million (14). However, the true prevalence is likely to be higher because of underdetection. Interestingly, one Asian individual with sitosterolemia was identified out of 2542 persons from Dallas, Texas, in whom plasma plant sterols were analyzed (15). Some populations, due to a founder effect, reveal a much higher carrier frequency of heterozygous disease-causing variants: up to 4% in the Old Order Amish, 8% in the North American Hutterites, and 13% in the inhabitants of Kosrae (Micronesia) (16).

The mainstay of therapy is dietary restriction of both cholesterol and plant sterols (vegetable oils, margarine, nuts, seeds, avocado, and chocolate) and the use of the sterol absorption inhibitor, ezetimibe, or bile acid sequestrants (16, 17). Certain shellfish (clams, oysters, scallops) contain a number of shellfish sterols that are also hyperabsorbed, and they should also be avoided (18). Although cholesterol restriction is not recommended for children under age 2 and pharmacotherapy is usually not performed for children under age 10, an individual with extremely high levels of cholesterol may begin therapy earlier (19). A low-fat/low-cholesterol diet was reported to be safe and effective in infants and children (20).

One of the key biochemical features of sitosterolemia is excessively reduced whole-body cholesterol synthesis (5). Honda et al (21) reported coordinate down-regulation of the entire pathway of cholesterol biosynthesis, including...
hepatic hydroxymethylglutaryl coenzyme A (CoA) reductase, acetoacetyl-CoA thiolase, and squalene synthase, in the liver and mononuclear leukocytes from patients with sitosterolemia. Yang et al (22) showed that stigmasterol, not sitosterol, inhibits processing of sterol regulatory-binding protein-2 (SREBP-2), a transcription factor involved in cholesterol biosynthesis, leading to reduced cholesterol synthesis in mice. It was also reported that stigmasterol and campesterol inhibit activation of SREBP-2 in cultured adrenocortical cells (23). It seems that these plant sterols, which are also excessively increased in patients with sitosterolemia, mimic the negative feedback effect on cholesterol synthesis.

Because homozygous FH patients are relatively refractory to dietary modification and cholesterol-lowering agents, the dramatic cholesterol reduction with diet and cholestyramine suggested alternative diagnoses such as sitosterolemia in our case. Plasma cholesterol levels in sitosterolemic patients are extremely sensitive to dietary cholesterol restriction and bile acid sequestrants, whereas they do not respond to statins (24).

In nonsitosterolemic individuals, cholesterol synthesis increases after sterol depletion, so the effect of sterol absorption inhibitor or bile acid sequestrants is limited (23). However, in those with sitosterolemia, there is no such compensatory increase in cholesterol synthesis (24), resulting in dramatic reduction in plasma cholesterol levels. Sitosterolemia should be suspected when the plasma cholesterol falls more than 40% on a low-cholesterol diet. Because hydroxymethylglutaryl CoA reductase activity is already maximally inhibited, it is not surprising that statins fail to reduce cholesterol levels in patients with sitosterolemia.

A significant phenotypic heterogeneity is seen in sitosterolemia, ranging from a 5-year-old girl who suffered from fatal myocardial infarction to a 75-year-old woman who was referred for evaluation of hyperlipidemia (4, 25). Our proband showed severe hypercholesterolemia and xanthomatosis, whereas her sibling was asymptomatic except for moderate hypercholesterolemia that was normalized after dietary modification. It is likely that different diet patterns especially during infancy may have resulted in different levels of plant sterols and cholesterol in these siblings, resulting in different phenotypes.

Sitosterolemia might be significantly underdiagnosed, especially in children in whom routine screening for lipid profiles is not usually performed. The new National Heart, Lung and Blood Institute guidelines recommend screening all children at 9–11 years and again at 17–21 years to find those with genetic conditions such as FH (26). Some of these children may in fact have sitosterolemia, and this group may be distinguished by either remarkable response to dietary modification or poor response to statins.

It is noteworthy that most pediatric patients with sitosterolemia show significantly elevated serum cholesterol levels that could be up to 1000 mg/dL, whereas adult patients tend to show only mild hypercholesterolemia (Table 1). The mechanism of exceptionally high cholesterol levels in sitosterolemic children is unclear as yet. It seems that in adults with sitosterolemia, increased levels of plant sterols, especially campesterol and stigmasterol, induce SREBP-mediated suppression of cholesterol synthesis. We hypothesize that in children, especially in breastfed infants whose plant sterol levels are not increased yet, this inhibitory mechanism could be incomplete, which may result in severe hypercholesterolemia.

To date, there are only two other reported cases of sitosterolemia that were diagnosed in breastfed infants. Rios et al (27) reported the case of an 11-month-old girl with xanthomatosis and striking hypercholesterolemia of
1023 mg/dL. Breast milk comprised 80% of her diet, and the sitosterol level was only slightly increased (2.37 mg/dL). Sitosterolemia was initially ruled out by documenting a normal plasma sitosterol:cholesterol ratio. The diagnosis of sitosterolemia was finally established by whole-genome sequencing, and markedly increased sitosterol levels were confirmed after weaning. Niu et al (6) reported a case of sitosterolemia in a 3-month-old girl with serum cholesterol levels of 402 mg/dL when she was being exclusively breastfed.

These cases, as well as the current one, describe unique features of breastfed infants with sitosterolemia. Human milk contains 90–150 mg/L of cholesterol, whereas infant formula contains 0–4 mg/L of cholesterol. The total cholesterol and LDL-C levels are higher in breastfed infants compared with formula-fed infants (28). The plasma plant sterol levels in heterozygotes are only slightly increased, and the only source of plant sterol in the breast milk is plasma (3). Probably, the plant sterol intake of a breastfed infant from the heterozygote mother would be much lower than on a diet based on fruits and vegetables. It is plausible that there is unopposed cholesterol synthesis that is not inhibited by plant sterols, as well as hyperabsorption and decreased biliary secretion of cholesterol, in breastfed sitosterolemic infants. The hypercholesterolemia seems to be somewhat relieved as they start taking food containing plant sterols. Measurement of serum plant sterol is regarded as the most reliable test for sitosterolemia, but this method might not be diagnostic in breastfed infants. In our case, the GC-MS was not available initially, and it was performed with the plasma drawn at 3 years of age. If the test had been performed at presentation, it would possibly have missed the diagnosis. We suggest that DNA sequencing of ABCG5/G8 should be performed to rule out sitosterolemia in infants.

Normal levels of serum cholesterol in the sitosterolemic patients may lead to a false reassurance, masking uncontrolled accumulation of plant sterols and the progression of atherosclerosis. In our patient, the GC-MS was performed when the patient’s LDL-C was controlled within the normal range, but the sitosterol level was extremely high. Her plant sterol intake was around 420 mg/d at the age of 56 months when estimated by a 24-hour recall method. Plant sterol intake may paradoxically increase during a low-saturated-fat/low-cholesterol diet. Foods rich in plant sterols are usually con-
sidered “healthy foods”, but not for patients with sitosterolemia.

It seems that plant sterols have direct atherogenic effect because some normocholesterolemic patients with sitosterolemia developed premature coronary heart disease. For instance, a 16-year-old sitosteremic girl with normal cholesterol levels was reported to have premature coronary heart disease requiring coronary bypass grafts (29), and a normocholesterolemic patient who underwent a three-vessel coronary bypass surgery at the age of 29 was diagnosed with sitosterolemia afterward (5). Plant sterols comprise 15 to 20% of total plasma sterols in patients with sitosterolemia and are carried in LDL and very low-density lipoprotein particles. Plant sterols are relatively poorly esterified by the sterol-esterifying enzyme acyl-CoA-cholesterol acyl transferase. Macrophages incubated with sitosterol-containing lipoproteins accumulated free sterols and underwent necrotic cell death, which may contribute to the formation of rupture-prone atherosclerotic plaque (30).

Most cases with sitosterolemia were accompanied by tendinous or tuberous xanthomas on extensor areas, such as elbows and knees (4, 13). However, planar xanthomas were observed in the intertriginous areas in our patient, suggesting that intertriginous xanthomas may develop in patients with extremely high LDL-C (>400 mg/dL) who have either homozygous FH or sitosterolemia.

Our case suggests that sitosterolemia should be suspected when a patient with hypercholesterolemia shows an unexpectedly good response to diet or bile acid sequestrant therapy, and when severe hypercholesterolemia is expected when a patient with hypercholesterolemia shows abnormalities of plasma sitosterol, apoprotein B, and lipoproteins in a large Amish pedigree with sitosterolemia. Familial homozygous hypercholesterolemia: report of two patients and review of the literature.

References

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