

Review article

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Sitosterolemia: a review and update of pathophysiology, clinical spectrum, diagnosis, and management

Eun-Gyong Yoo, MD, PhD

Department of Pediatrics,
CHA Bundang Medical Center,
CHA University, Seongnam, Korea

Sitosterolemia is an autosomal recessive disorder characterized by increased plant sterol levels, xanthomas, and accelerated atherosclerosis. Although it was originally reported in patients with normolipemic xanthomas, severe hypercholesterolemia have been reported in patients with sitosterolemia, especially in children. Sitosterolemia is caused by increased intestinal absorption and decreased biliary excretion of sterols resulting from biallelic mutations in either ABCG5 or ABCG8, which encode the sterol efflux transporter ABCG5 and ABCG8. Patients with sitosterolemia show extreme phenotypic heterogeneity, ranging from almost asymptomatic individuals to those with severe hypercholesterolemia leading to accelerated atherosclerosis and premature cardiac death. Hematologic manifestations include hemolytic anemia with stomatocytosis, macrothrombocytopenia, splenomegaly, and abnormal bleeding. The mainstay of therapy includes dietary restriction of both cholesterol and plant sterols and the sterol absorption inhibitor, ezetimibe. Foods rich in plant sterols include vegetable oils, wheat germs, nuts, seeds, avocado, shortening, margarine and chocolate. Hypercholesterolemia in patients with sitosterolemia is dramatically responsive to low cholesterol diet and bile acid sequestrants. Plant sterol assay should be performed in patients with normocholesterolemic xanthomas, hypercholesterolemia with unexpectedly good response to dietary modifications or to cholesterol absorption inhibitors, or hypercholesterolemia with poor response to statins, or those with unexplained hemolytic anemia and macrothrombocytopenia. Because prognosis can be improved by proper management, it is important to find these patients out and diagnose correctly. This review article aimed to summarize recent publications on sitosterolemia, and to suggest clinical indications for plant sterol assay.

Keywords: Sitosterolemia, Phytosterolemia, Hypercholesterolemia, Plant sterol

Introduction

Sitosterolemia, also known as phytosterolemia, is an autosomal recessive disorder characterized by increased plant sterol levels, xanthomas, and accelerated atherosclerosis^{1,2}. It is caused by increased intestinal absorption and decreased biliary excretion of plant sterols resulting from homozygous or compound heterozygous mutations in either ABCG5 or ABCG8, which encode the sterol efflux transporter ABCG5 (sterolin-1) and ABCG8 (sterolin-2) that pumps sterols out to intestinal lumen or into bile^{3,4}. Although it is a rare disease, it is an important disease that led to understanding of the physiologic pathway about sterol influx and efflux^{5,6}.

Mediterranean stomatocytosis/macrothrombocytopenia has been identified as the hematologic presentation of sitosterolemia⁷. Stomatocytic hemolysis, large platelets, splenomegaly, and abnormal bleeding can be associated, and hematologic manifestations can be the only clinical sign of sitosterolemia⁸. The true prevalence of sitosterolemia is unknown due to

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Address for correspondence:

Eun-Gyong Yoo, MD, PhD
Department of Pediatrics, CHA
Bundang Medical Center, CHA
University, 59 Yatap-ro, Bundang-gu,
Seongnam 13496, Korea
Tel: +82-31-780-1999
Fax: +82-31-780-5239
E-mail: pedyoo@cha.ac.kr

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underdiagnosis, and sitosterolemia may be more frequent than previously thought. One Asian individual with sitosterolemia was identified incidentally out of 2,542 persons from a study in which plasma plant sterols were analyzed⁹.

Because delayed diagnosis can lead to poor clinical outcome due to advanced atherosclerotic cardiovascular disease and prognosis can be improved by proper management including plant sterol restriction and cholesterol absorption inhibitor in sitosterolemia, it is important to find these patients out and diagnose correctly^{2,8,10}. This review article aimed to summarize recent publications on the pathophysiology, clinical spectrum, diagnosis and management of sitosterolemia, and to suggest clinical indications for plant sterol assay.

The plant sterols

Plant sterols are rich in vegetable oils, wheat germs, nuts, seeds, avocados, chocolate, and margarine^{2,6}. They are structurally very similar to cholesterol, but they differ by the presence of an ethyl or methyl group (sitosterol and campesterol, respectively) or a double bond (stigmasterol)¹. Sitosterol is usually the most abundant plant sterol in the diet and the predominant form found in patients with sitosterolemia^{8,11}.

Average Western diet contains similar amount of cholesterol and plant sterols. Although approximately 50% of dietary cholesterol is absorbed, less than 5% of plant sterols are absorbed in normal individuals^{6,11}. High plant sterol diet was extremely toxic in animal models of sitosterolemia, and it was suggested that the mammalian body defends itself against plant sterols because they are toxic when accumulated, although similar toxicity have not been documented in human yet^{12,13}.

It is clear that plant sterols are toxic to those with sitosterolemia, but plant sterol intake seems to be safe to nonsitosterolemic individuals. Instead, plant sterols can competitively inhibit cholesterol absorption, and the cholesterol lowering effect of plant sterols have been documented¹¹. Although they have not been shown to reduce clinical outcomes, many cholesterol lowering functional foods are enriched with plant sterols^{14,15}. On the other hand, studies have raised the possibility of association between plant sterol levels and atherosclerosis^{16,17}. The cholesterol lowering effect may compensate the potential risk of increased plant sterol intake^{9,11}, and the debate whether plant sterol is beneficial or harmful is still ongoing^{15,18}.

Sterol absorption in normal subjects

Dietary cholesterol and noncholesterol sterols, mainly plant sterols and stanols (saturated sterols), are absorbed from the intestinal lumen via the sterol influx transporter, Nieman Pick C1 Like 1 (NPC1L1)⁶. The NPC1L1, the gatekeeper of sterol absorption, have lower affinity to plant sterols than cholesterol^{19,20}. After absorption to the enterocytes, about 50%–60% of cholesterol is esterified by the acetyl-sterol O-acetyltransferase 2 (SOAT2) and transported to liver packed in the

chylomicrons²¹. Unesterified cholesterol or plant sterols are pumped back to intestinal lumen by ABCG5/ABCG8, the sterol efflux transporters⁶. The SOAT2 also have low affinity with plant sterols, allowing preferential plant sterol efflux by the ABCG5/ABCG8²². Plant sterols not pumped back to the intestinal lumen become part of the chylomicrons, transported to the liver, and eventually pumped out into the bile by the hepatic ABCG5/ABCG8 transporters^{6,11}.

Disrupted sterol homeostasis in sitosterolemia

Biallelic defects in either *ABCG5* or *ABCG8* result in increased intestinal absorption and decreased biliary excretion of plant sterols, leading to extremely high plasma levels of plant sterols^{3,4}. Patients with sitosterolemia absorb 15% to 60% of ingested sitosterol, which lead to a 50- to 200-fold increase in their plasma sitosterol levels^{1,3}. Plant sterols comprise 15% to 20% of total plasma sterols in patients with sitosterolemia and are carried in low-density lipoprotein (LDL) and very-LDL particles^{3,23}.

In a patient with liver failure and sitosterolemia that underwent liver transplantation, the elevated plant sterol levels decreased to values less than 1/10 of pretransplantation level, suggesting that the liver functions as the predominant organ for maintaining sterol balance²⁴. *ABCG5/ABCG8* expression either in liver or intestine protected animals from sterol accumulation in a recent study²⁵.

Although sterol absorption was moderately increased in heterozygotes, they are asymptomatic with normal cholesterol levels and normal to slightly increased plant sterol levels^{1,26}.

Clinical spectrum of sitosterolemia

Patients with sitosterolemia show extreme phenotypic heterogeneity. Whereas some patients with homozygous mutations are almost totally asymptomatic, others show severe hypercholesterolemia leading to accelerated atherosclerosis and premature cardiac death^{1,27-30}. A 10-year-old girl from Iran, who had received almost vegetable-free diet in Iran and started to intake much more vegetables and olive oil after her family moved to Europe, developed xanthomas and hypercholesterolemia in a short period of time and was finally diagnosed for sitosterolemia³¹. Although the amount of dietary plant sterol intake should be at least partially related with the severity of clinical disease, the mechanism of phenotypic heterogeneity, even between the family members that shares same gene and environment, is not fully understood yet. A recent report in a Chinese family with sitosterolemia suggested potential effects of NPC1L1 polymorphisms in protecting against clinical disease²⁹. Major clinical features of sitosterolemia, especially in young patients with sitosterolemia are summarized in Table 1.

Table 1. Clinical spectrum of sitosterolemia

Clinical spectrum of sitosterolemia	References
Asymptomatic (with or without hypercholesterolemia)	28-30
Xanthomas (with or without hypercholesterolemia)	
Tendinous or tuberous xanthomas on extensor sites	1,10,23,27,28
Planar xanthomas in the creases of Achilles region	33
Intertriginous xanthomas	35
Hypercholesterolemia	
Mild to moderate hypercholesterolemia	10,28,31,44
Severe hypercholesterolemia (≥ 500 mg/dL)	32,33,35,47
Premature cardiovascular disease	
Premature coronary heart disease (at age 16–29)	10,29
Sudden cardiac death (at age 5–18)	22,47,48
Hematologic manifestations	
Hemolytic anemia with stomatocytosis	7,8,52,54
Macrothrombocytopenia	7,8,52,54
Splenomegaly	8,53
Abnormal bleeding	55
Arthritis, arthralgia	2
Hepatic failure*	24

*Not confirmed whether hepatic failure was due to sitosterolemia or not.

1. Hypercholesterolemia

Although it was originally reported in patients with normolipemic xanthomas²⁷, cholesterol absorption is also increased in patients with sitosterolemia, and serum cholesterol levels are usually elevated^{1,10}. Very high levels of cholesterol (up to 1,000 mg/dL) have been reported in patients with sitosterolemia, especially in children^{32,33}. Immature intestine may absorb higher amounts of cholesterol compared with that of adults³⁴.

Breastfed infants with sitosterolemia show unique clinical features^{32,33,35}. The plant sterol intake of a breastfed infant should be minimal because the plasma sitosterol levels of the heterozygote mother should be only slightly increased. However their cholesterol intake can be high due to high cholesterol content of human milk (90–150 mg/L vs. 0–4 mg/L in human milk and infant formula, respectively)³⁶. Breastfed infants with sitosterolemia can present with extremely high cholesterol levels with xanthomatosis, but with normal sitosterol:cholesterol ratio due to only mildly elevated plant sterol levels³³. The plant sterol level increase and the cholesterol level somewhat decrease as the infant start taking fruits and vegetables^{33,35}.

2. Xanthomas

Tendinous or tuberous xanthomas on extensor areas, such as Achilles tendon, extensor tendons of the hand, elbows and knees are the major clinical manifestations of sitosterolemia^{1,23,27}. Minor trauma plays an important role in the development of xanthomas, and this is why they appear on extensor surfaces in most patients³⁷.

Xanthomatosis is rarely observed in young children, and when present, homozygous familial hypercholesterolemia (FH) or autosomal recessive hypercholesterolemia is most often suspected^{37,38}. Xanthomas may begin to appear at very young age in sitosterolemia, sometimes during the first year of life^{33,35}.

Intertriginous xanthomas are a very rare type of planar xanthomas and have been reported to be pathognomonic for homozygous FH³⁹. However, intertriginous xanthomas (first noticed at the age of 3 months when the patient was being exclusively breastfed) were observed in a 15-month-old Korean girl with sitosterolemia, suggesting that intertriginous xanthomas may develop in young children with extremely high cholesterol levels of any etiology³⁵. Friction between the skins in intertriginous areas may contribute to the development of xanthomas in a chubby infant in whom extensor areas are relatively spared because movement is not active yet.

Most sitosterolemic patients with severe atherosclerotic cardiovascular disease also showed xanthomas^{1,10,23,28}. Xanthomas evolve as clusters of foam cells in the skin, and the mechanisms involved in the development of xanthoma seem to be similar to those in early stages of atherosclerotic plaques⁴⁰. According to a meta-analysis on patients with genetic diagnosis of FH, the presence of tendon xanthomas was associated with a 3.2 times higher risk of cardiovascular disease⁴¹. Xanthelasma of the eyelids was considered to be only a cosmetic lesion until recently, however recent prospective studies showed that it is connected with an increased cardiovascular risk and reduced average lifespan⁴². In contrast to the initial case with normolipemic xanthomas²⁷, xanthomas regress and sometimes completely disappear in some patients with sitosterolemia, usually associated with dramatically decreased plasma cholesterol levels, although plant sterol levels were still relatively high^{32,35,43-46}.

3. Atherosclerotic cardiovascular disease

Some patients with sitosterolemia develop premature atherosclerosis leading to sudden cardiac death at as early as 5⁴⁷, 13⁴⁸, 18²² years of age, whereas others, even in the same family of symptomatic patients, do not show any classic sign of sitosterolemia^{24,28,35}.

Both elevated plasma cholesterol and plant sterol levels can contribute to the premature vascular disease in patients with sitosterolemia. Accumulation of plant sterol in plasma lipoproteins influences the stability of both cholesterol and plant sterol in lipoproteins, favoring the accumulation of these sterols within tissues, initiating inflammatory reactions, and may cause premature atherosclerosis⁶.

Coronary plaque disruption and superimposed thrombosis is the major cause of acute myocardial infarction and sudden cardiac death⁴⁹. The composition and vulnerability of plaque rather than its volume or the severity of stenosis are more important for the development of the thrombus-mediated acute coronary syndromes⁴⁹. 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 plaque⁵⁰.

Premature coronary heart disease can develop in sitosterolemic patients with normal cholesterol levels. A 16-year-old sitosterolemic girl with normal cholesterol level was reported to have premature coronary heart disease requiring coronary bypass grafts⁵¹, and a normocholesterolemic patient who underwent a 3 vessel coronary bypass surgery at the age of 29 was diagnosed with sitosterolemia after that¹⁰.

On the other hand, Hansel et al.³⁰ could not find significant signs of premature atherosclerosis in 5 patients with sitosterolemia aged 11 to 21 years, in spite of severe hypercholesterolemia as well as extremely high plant sterol levels. They suggested that the premature atherosclerosis in some patients with sitosterolemia may be due at least in part to mechanisms independent of elevated circulating plant sterol levels³⁰.

4. Hematologic manifestations

Rees et al.⁷ revealed that stomatocytic hemolysis and macrothrombocytopenia (previously known as the Mediterranean stomatocytosis or Mediterranean macrothrombocytopenia, which had been a poorly understood hematological condition) is the hematological presentation of sitosterolemia.

Stomatocytosis, hemolytic anemia, thrombocytopenia with very large platelets, splenomegaly, and abnormal bleeding can be associated with sitosterolemia⁸. Because the *ABCG5* and *ABCG8* are only expressed in intestine and liver, acquired accumulation of circulating plant sterols and their incorporation into red blood cells (RBC) and platelet seems to be resulting in abnormal morphology and function⁷.

Blood cells can be a main target for the toxic effect of plasma plant sterols, and sitosterolemia can be manifested mainly by hematologic abnormalities⁵². Three patients from a Chinese family, all of whom had suffered from severe hemolytic anemia and macrothrombocytopenia since 3 to 4 years of age and underwent splenectomy in their 10's, was diagnosed as sitosterolemia in their 20's. All of these patients had increased plasma sitosterol but normal cholesterol levels⁵². Thirteen sitosterolemic patients with hematologic manifestations, including 2 patients without any classical features of sitosterolemia, had been misdiagnosed with immune thrombocytopenia (ITP), Evans syndrome, or secondary ITP with delay being 15 to 49 years between symptom onset and correct diagnosis⁵³. Plasma plant sterols should be analyzed in patients with unexplained hemolytic anemia with macrothrombocytopenia to avoid unnecessary splenectomy⁵⁴.

Recently, Kanaji et al.⁵⁵ have identified that the bleeding abnormalities and macrothrombocytopenia associated with sitosterolemia are due to direct plant sterol incorporation into the platelet membrane, resulting in platelet hyperactivation,

reduced α IIb β 3 surface expression, loss of the GPIIb-IIIa linkage, microparticle formation, and ultimately poor hemostatic functions.

Diagnosis of sitosterolemia

Routine laboratory methods do not distinguish plant sterols from cholesterol, and a more accurate method such as gas chromatography-mass spectrometry (GC-MS) is required. Measurement of serum plant sterol by GC-MS or liquid chromatography-mass spectrometry is regarded as a reliable test for screening sitosterolemia, in which unequivocally increased plant sterol levels and sitosterol: cholesterol levels are almost invariably observed¹.

Genetic confirmation can be given by direct sequencing of exons and intron-exon boundaries of the *ABCG5* and *ABCG8* genes, each comprised of 13 exons and located in a head-to-head organization on chromosome 2p21, and documenting the homozygous or compound heterozygous mutations in either *ABCG5* or *ABCG8*^{3,4}. Asian patients usually have mutations in *ABCG5*, while Caucasian patients usually have *ABCG8* mutations^{3,32}. However, mutations in *ABCG8* have been reported in 3 of 8 families with hematologic manifestations of sitosterolemia according to a recent Chinese study, suggesting that *ABCG8* mutations are not exclusive to Caucasians⁵³. DNA sequencing of *ABCG5/ABCG8* is should be performed to rule out sitosterolemia in breastfed infants, because they can exhibit only mild elevation of plasma sitosterol level and normal sitosterol:cholesterol ratio³³.

In contrast to patients with homozygous FH that are relatively refractory to dietary modification and cholesterol-lowering agents, plasma cholesterol levels in sitosterolemic patients are extremely sensitive to dietary cholesterol restriction and bile acid sequestrants^{32,43,44}.

The entire pathway of cholesterol biosynthesis including hepatic hydroxymethylglutaryl coenzyme A (HMG CoA) reductase is exceptionally down-regulated in patients with sitosterolemia^{10,56}. It was also reported that stigmasterol and campesterol inhibit activation of sterol regulatory binding protein-2 (SREBP-2), a transcription factor involved in cholesterol biosynthesis, in cultured adrenocortical cells⁵⁷, and that stigmasterol, not sitosterol, inhibits processing of SREBP-2 leading to reduced cholesterol synthesis in mice⁵⁸.

In nonsitosterolemic individuals, cholesterol synthesis increases after sterol depletion, limiting the effect of sterol absorption inhibitor or bile acid sequestrant⁵⁷. However, there is no such compensatory increase in cholesterol synthesis in those with sitosterolemia, resulting in dramatic reduction in plasma cholesterol levels⁵⁹. Sitosterolemia should be suspected when the plasma cholesterol falls more than 40% on a low-cholesterol diet.

Sitosterolemia seems to be significantly underdiagnosed, and many of these patients should be continuing to intake large amount of plant sterols, not knowing that the plant sterols are 'toxic' to them, but believing that those food are

good for their health. Sitosterolemia might also be significantly underdiagnosed in children in whom screening for lipid profiles is not universally performed. Recent guidelines recommend screening all children at 9–11 years and again at 17–21 years to find those with hypercholesterolemia⁶⁰. Some of those screened may in fact have sitosterolemia, and these patients may be distinguished by either remarkable response to dietary modification or poor response to statins³⁵.

Management of sitosterolemia

Management of sitosterolemia aims to reduce plasma plant sterol (as low as possible; although perfect control [sitosterol level <1 mg/dL] cannot be achieved) and cholesterol concentrations and to prevent or reduce xanthomas and atherosclerotic cardiovascular diseases².

Mainstay of therapy is dietary restriction of both cholesterol and plant sterols. Foods rich in plant sterols include vegetable oils, wheat germs, nuts, seeds, avocado, most of which are known to be heart-healthy foods^{2,61}. Margarine, shortening, and chocolate should also be avoided. Polished rice should be taken instead of whole grains. Shellfish and seaweeds contain significant amount of algae-derived plant sterols that are also hyperabsorbed in these patients, and they should also be avoided⁶². However, plant sterol-free diet is almost impossible to accomplish because plant sterols are found in almost every plant-based foods, and low plant sterol diet have resulted in only about 30% reduction of plasma plant sterol levels^{11,44}.

Pharmacotherapy include the sterol absorption inhibitor, ezetimibe, or bile acid sequestrants such as cholestyramine. Patients with sitosterolemia usually do not respond to statins because HMG CoA reductase activity is already maximally inhibited⁶⁰.

The bile acid sequestrants inhibit the reabsorption of bile acids in the ileum and disrupt the enterohepatic circulation of bile acids. The bile acid sequestrants was reported to reduce plasma plant sterol levels by up to 45%, although they may result in more dramatic decrease in plasma cholesterol levels (50%–80%) and regression of xanthomas^{43,44}. Sitosterolemia should be considered in patients with hypercholesterolemia and/or xanthomas who show dramatic reduction of cholesterol levels or regression of xanthomas by bile acid sequestrant therapy. However, poor compliance and gastrointestinal side effects limit the use of cholestyramine.

Ezetimibe, an inhibitor of intestinal sterol absorption

through its binding to NPC1L1, is currently considered the choice of treatment for sitosterolemia⁶³. It has been widely used for decreasing serum LDL-cholesterol levels in patients with hypercholesterolemia. Ezetimibe also reduces the intestinal absorption of plant sterols, thereby also lowering plasma plant sterol levels. Ezetimibe alone or in combination with cholestyramine successfully decreased plasma cholesterol and plant sterol levels (about by 50%; although still much higher than normal values)⁶³, resulting in regression of xanthomas and improvement of carotid bruits and cardiac murmurs in patients with sitosterolemia⁴⁵. Long-term treatment with ezetimibe 10 mg/day was safe, tolerable, and effective in reducing plasma plant sterol concentrations in patients with sitosterolemia^{61,64}. Ezetimibe reduced plasma and RBC plant sterol levels, while increasing platelet count and decreasing mean platelet volume, and thereby may reduce the risk for bleeding in sitosterolemia⁶⁵.

Although pharmacotherapy is usually not performed for children under age 10, an individual with extremely high levels of cholesterol may begin therapy earlier⁶⁶. Ezetimibe therapy seems to be also safe and effective in children with sitosterolemia, although an infant did not respond to ezetimibe therapy at 7 months of age possibly due to immature glucuronidation system, who finally showed improvement when ezetimibe was restarted at 2 years of age³². Bile acid sequestrants such as cholestyramine can be added for those with insufficient response to ezetimibe^{2,63}.

Arthritis and arthralgia can also be associated with sitosterolemia, and more strict management of sitosterolemia can be helpful².

Conclusions

Plant sterol assay should be performed in patients with normocholesterolemic xanthomas, hypercholesterolemia with unexpectedly good response to dietary modifications or to cholesterol absorption inhibitors, or hypercholesterolemia with poor response to statins, or those with unexplained hemolytic anemia and macrothrombocytopenia (Table 2).

The dramatic cholesterol reduction and regression of xanthomas by proper treatment including plant sterol restriction and cholesterol absorption inhibitor suggest that sitosterolemia can be a controllable condition, and it is important to find these patients out and diagnose correctly because prognosis can be improved by early diagnosis and proper management.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Table 2. Indications for plant sterol assay

Indications for plant sterol assay
Normocholesterolemic xanthomas
Dramatic decrease of cholesterol levels in response to low cholesterol diet and/or bile acid sequestrants or cholesterol absorption inhibitors
Regression of xanthomas, especially with dramatically improved cholesterol levels
Hypercholesterolemia with poor response to statins
Unexplained hemolytic anemia with macrothrombocytopenia

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