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Case Report

Long-Term Follow-Up of a Patient with Sitosterolemia and Hemolytic Anemia with Excellent Response to Ezetimibe

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Sitosterolemia (MIM #210250), also known as phytosterolemia, first described in 1974, is characterized by disruption of the normal homeostatic mechanisms that regulate dietary cholesterol absorption and prevent the accumulation of non-cholesterol sterols [1]. Phytosterols are almost undetectable in plasma from normal individuals [2]. Sitosterolemia is inherited in an autosomal recessive fashion and results from mutations in two genes at the *STSL* locus that maps to human chromosome 2p21: the ATP-binding cassette, subfamily G, members 5 and 8 (*ABCG5* and *ABCG8*), that encode two proteins known as sterolin-1 and -2 [3,4]. Since the expression of these genes is strictly restricted to the intestinal epithelium and the liver, it is believed that the proteins encoded by *ABCG5* and *ABCG8* function as sterol efflux pumps. However, the relative importance of the liver and the intestine in maintaining sterol balance with respect to non-cholesterol sterols is unknown. Patients with sitosterolemia accumulate high concentrations of plant sterols in plasma and tissues that leads inexorably to the development of subcutaneous and tendon tuberoeruptive xanthomas during the first years of life and occasionally to the development of xanthelasmas, arcus corneae, and premature coronary artery disease. The development of hematologic abnormalities in patients with sitosterolemia, most importantly hemolysis and macrothrombocytopenia, is occasionally seen and their management is unknown [1]. Herein, we describe the long-term follow-up of a case of sitosterolemia associated with hemolytic anemia, successfully managed with lipid lowering therapy.

A 9-month-old Caucasian baby developed yellow streaks on her wrists. By 14 months of age, she underwent a skin biopsy of a right wrist lesion, which revealed foamy histiocytes compatible with xanthomas. At that time, her serum total cholesterol was 802 mg/dL, triglycerides were 190 mg/dL, and HDL was 32 mg/dL. All developmental milestones were achieved at appropriate ages. The patient was placed on a very strict low-fat diet that resulted in normalization of her total cholesterol and triglyceride levels. Interestingly, her family history was notable for a maternal grandmother with history of elevated cholesterol and xanthomas that developed at age 36 and resolved with minor dietary modifications. In addition, her paternal grandmother had developed xanthelasmas and her maternal grandfather had

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atherosclerosis that led to the development of multiple myocardial infarctions by age 55. The patient's brother had no medical problems. At age 5, the patient underwent an extensive lipid work-up that demonstrated the presence of a normal total cholesterol level but an elevated sitosterol level at 30.7 mg/dL (Table 1 and Figure 1). Since then, her diet was implemented to avoid all oils except soy, as well as chocolate, nuts, and legumes. The strict diet notwithstanding, at 12 years of age the patient developed *arcus juvenilis* and by 13 years of age she presented with enlarging tendon and tuberous xanthomas that began on her knuckles and progressed to affect both elbows and Achilles tendons. She was also started on therapy with the bile acid binding drug colesevelam hydrochloride.

By 16 years of age, the patient was referred to our hematology clinic for evaluation of pancytopenia. On physical exam, she had xanthomas in both elbows and hepatomegaly and splenomegaly palpable 2 cm and 5 cm below the costal margin, respectively. Her initial peripheral blood cell count revealed white blood cell (WBC) count 3.4x10^9/L (neutrophils 58%, bands 7%, lymphocytes 31%, monocytes 2%, eosinophils 1%, basophils 1%), platelet count 83x10^9/L, hemoglobin 10.1 g/dL, and median corpuscular volume (MCV) 90 fL. Despite a very low serum ferritin level (18 ng/mL), her retyculocyte count was 7%. The peripheral blood smear exam revealed neutrophilic vacuolation, normocytic normochromic anemia with anisopoikilocytosis, and mild thrombocytopenia with frequent large platelets. Hepatitis B and C, HIV serologies, anti-nuclear antibodies, rheumatoid factor, and thyroid function tests were either negative or within normal limits. Both, hemoglobin and protein serum electrophoreses were normal. After 3 months on ferrous gluconate her hemoglobin only increased to 10.8 g/dL. The coding regions and intron/exon boundaries of *ABCG5* and *ABCG8* of the patient and her first degree relatives were amplified and sequenced as previously described [4]. This analysis revealed the patient to carry wild-type *ABCG5* but mutant *ABCG8* alleles: Trp361X (1173G→A)/Tyr658X (2064C→G).

Subsequently, the patient was lost to follow-up but 2 years later she presented again to our clinic with a WBC 4.8x10^9/L, hemoglobin 7.6 g/dL, and platelet count 83x10^9/L. At that time, there was evidence of hemolysis with a retyculocyte count of 7.1%, bilirubin 1.5 mg/dL (indirect 1.3 mg/dL), lactate dehydrogenase (LDH) 725 U/L, and haptoglobin <6 mg/dL. A peripheral blood film revealed the presence of abnormally shaped erythrocytes with a moderate number of spherocytes, polychromasia, and macrothrombocytopenia. The serum ferritin level was <6 ng/mL and a bone marrow aspiration and biopsy revealed normal cellularity, increased erythroid precursors, no blast hyperplasia, and markedly decreased iron stores. Iron supplementation was again resumed with no significant improvement in hemoglobin levels. Remarkably, red cell studies showed a decreased erythrocyte osmotic fragility. The results of other routine hematologic tests to identify the cause of hemolysis were unremarkable, including Coombs' test, Ham's test, and glucose-6-phosphate dehydrogenase.

At age 23, routine blood tests demonstrated elevation of transaminases with ALT 321 U/dL and AST 144 U/dL. Given the

Table 1: Levels of serum lipids and plasma phytosterols over time. Fasting blood samples were collected into test-tubes without anticoagulants from the patient. Determination of β-sitosterol, sitostanol, campesterol, campestanol, and cholestanol were quantified by high-performance liquid chromatography (HPLC), as previously described.

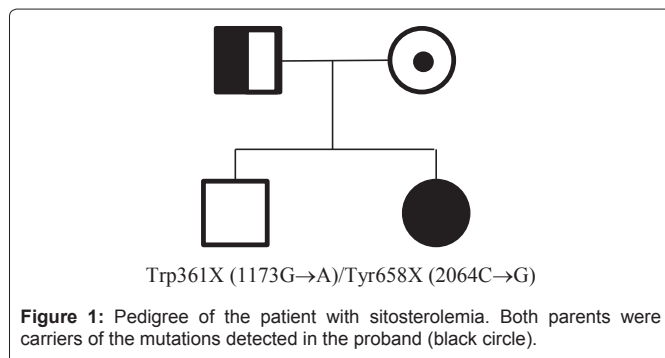| Patient age (years)	Total Ch (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	cholestanol (mg/dL)	campesterol (μg/dL)	campestanol (μg/dL)	sitosterol (mg/dL)	sitostanol (mg/dL)
5	291	92	56			13		30.7	
7	271	81	65						
8	255	104	53						
9	258	88	63						
10	257	83	59						
11	263	84	47	199					
12	223	100	54	149					
13	200	88	59	123		11		24	
16	134				1	15	2.5	32	4.4
17						14.6		30	
20	214				1.95	18.5	1.2	40	12
23	257	92	68	151	1.95	18.5	1.2	30	12
25	286	93	49	218					
26	171	56	64	95					
Normal range	**100-199**	**0-149**	**40-59**	**5-40**	**0.25-0.45**	**1.5-9.7**	**0.02-0.08**	**0.3-1**	**2-4.80**

inability of increasing doses of colesevelam to control her cholesterol and sitosterol levels, therapy with ezetimibe 10 mg daily was started. After 2 years on ezetimibe, her cholesterol levels were still high but her transaminase levels returned to normal and all signs of hemolysis disappeared, including normalization of the hemoglobin level, red cell morphology, reticulocyte count, bilirubin, and LDH levels. However, phytosterol levels remained high, with only a mild reduction in campestanol being observed. Therapy with a combination of ezetimibe and simvastatin was then started, which partially corrected her cholesterol levels.

Sterols are non-cholesterol sterols that along with phospholipids constitute the main components of cell membrane, being crucial for ions and metabolites transport as well as cell permeability and rigidity [5]. While cholesterol is preferentially absorbed in the intestinal tract by the sterol transporter Niemann-Pick C1L1, phytosterols are preferentially extruded by ABCG5/ABCG8 transporters [6]. As a consequence, phytosterol concentration in human plasma is less than 0.5% that of cholesterol, despite similar ingestion [7]. As a result, plant sterols accumulate in all tissues but the brain, manifesting as xanthomas and premature atherosclerosis [8]. Given the critical

role that membrane flexibility plays in erythrocyte homeostasis and the high content of phytosterols on their surface in sitosterolemia, some patients with this disorder develop hemolysis [9]. The patient herein presented, who was followed for more than 25 years from birth, exemplifies this potential complication. Interestingly, anemia was observed at age 16, and became severe by 18 years of age, with unequivocal signs of hemolysis. While the mechanism of action whereby ezetimibe might improve hemolysis is currently unknown, it is tempting to speculate that therapy with this agent may modulate the lipid content at the red cell, thus increasing its resistance to lysis. Interestinly, a recent case report disclosed similar peripheral blood manifestations (i.e. thrombocytopenia and hemolysis) in a 31 year old woman with mutations at ABCG5 [10]. Therapy with ezetimibe progressively corrected the manifestations of hemolysis over a period of 24 months. Therapies for sitosterolemia such as bile salt-binding resins or HMG-CoA reductase inhibitors are largely ineffective [7]. Recently, ezetimibe, a selective inhibitor of the NPC1L1 intestinal sterol transporter, has been shown to block the transport of dietary cholesterol and phytosterols and to progressively reduce the levels of sitosterol and campesterol, the predominant plant sterols [11]. Notably, in our case, no significant decline in the levels of these plant sterols was observed. Yet, all markers of hemolysis and liver dysfunction normalized during ezetimibe therapy, while platelet counts remain low and unchanged, suggesting that this agent may improve some but not all features of the phenotype present in patients with sitosterolemia. Long-term follow-up is warranted to fully assess the ability of ezetimibe to further reduce plasma and tissue phytosterol levels.

References

1. Bhattacharyya AK, Connor WE (1974) Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. J Clin Invest 53:1033-1043.

2. Patel SB, Salen G, Hidaka H, Kwiterovich PO, Stalenhoef AF, et al. (1998) Mapping a gene involved in regulating dietary cholesterol absorption. The

Figure 1: Pedigree of the patient with sitosterolemia. Both parents were carriers of the mutations detected in the proband (black circle).

Trp361X (1173G→A)/Tyr658X (2064C→G)

sitosterolemia locus is found at chromosome 2p21. J Clin Invest 102: 1041-1044.

3. Berge KE, Tian H, Graf GA, Yu L, Grishin NV, et al. (2000) Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. Science 290: 1771-1775.

4. Lu K, Lee MH, Hazard S, Brooks-Wilson A, Hidaka H, et al. (2001) Two genes that map to the STSL locus cause sitosterolemia: genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by ABCG5 and ABCG8, respectively. Am J Hum Genet 69: 278-290.

5. Boesze-Battaglia K, Schimmel R (1997) Cell membrane lipid composition and distribution: implications for cell function and lessons learned from photoreceptors and platelets. J Exp Biol 200: 2927-2936.

6. Altmann SW, Davis HR Jr, Zhu LJ, Yao X, Hoos LM, et al. (2004) Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. Science 303:1201-1204.

7. Izar MC, Tegani DM, Kasmas SH, Fonseca FA (2011) Phytosterols and phytosterolemia: gene-diet interactions. Genes Nutr 6: 17-26.

8. Salen G, Horak I, Rothkopf M, Cohen JL, Speck J, et al. (1985) Lethal atherosclerosis associated with abnormal plasma and tissue sterol composition in sitosterolemia with xanthomatosis. J Lipid Res 26: 1126-1133.

9. Solca C, Stanga Z, Pandit B, Diem P, Greeve J, et al. (2005) Sitosterolaemia in Switzerland: molecular genetics links the US Amish-Mennonites to their European roots. Clin Genet 68: 174-178.

10. Wang G, Wang Z, Liang J, Cao L, Bai X, et al. (2011) A phytosterolemia patient presenting exclusively with macrothrombocytopenia and stomatocytic hemolysis. Acta Haematol 126: 95-98.

11. Salen G, von Bergmann K, Lütjohann D, Kwiterovich P, Kane J, et al. (2004) Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. Circulation 109: 966-971.

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