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Highlights:

- Sitosterolemia can be a multi-system disorder and is easily misdiagnosed.
- Abnormal blood cell morphology is relatively distinctive feature of sitosterolemia.
- Gene testing and plant sterols analysis help confirm a diagnosis of sitosterolemia.
Case study

Misdiagnosis of sitosterolemia in a patient as Evans syndrome and familial hypercholesterolemia

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Abstract

Sitosterolemia is a rare form of dyslipidemia that has diverse clinical manifestations, and insufficient knowledge of the disease frequently leads to a delay in diagnosis. We report a case of sitosterolemia in a 26-year-old Chinese woman, characterized by anemia, thrombocytopenia, persistent hypercholesterolemia, premature atherosclerosis, extensive xanthoma, and arthralgia-tenosynovitis. Successive misdiagnoses of Evans syndrome and familial hypercholesterolemia had been made, and the patient had responded minimally to steroid therapy, splenectomy, and statin treatment; therefore, she was referred to our hospital. On admission, a peripheral blood smear revealed the presence of abnormally shaped erythrocytes and giant platelets. Multiple atherosclerotic lesions, sites of tenosynovitis, and carotid sheath xanthomas were identified on ultrasonography. Compound heterozygous mutations of the ABCG5 gene, including a hot variant (c.1,336, exon10 C>T, p.(R446*)) and a novel variant (c.1,325-3(IVS9)_c.1325-2(IVS9)delCA) were separately identified in her parents by pedigree analysis. Plant sterols analysis by high performance liquid chromatography method revealed remarkably elevated plasma plant sterol concentrations after drug withdrawal but reduced rapidly after restarting ezetimibe during follow-up period. After 21 months of treatment with ezetimibe and a low-plant sterol diet, her hematologic abnormalities, tenosynovitis, and hypercholesterolemia had significantly improved; and ultrasonography showed that her skin and carotid sheath xanthomas had resolved or shrunk. This case demonstrates that morphological changes in blood cells on a peripheral blood smear, ultrasonographic findings and ABCG5/ABCG8 gene screening are valuable, and plant sterol
analysis in serum is crucial to confirm diagnosis and assess treatment adequacy for sitosterolemia.

**Key words**
Sitosterolemia, anemia, macrothrombocytopenia, xanthomas, atherosclerosis, hypercholesterolemia, tenosynovitis, ABCG5, misdiagnosis
Introduction

Sitosterolemia is a rare autosomal recessive disorder (approximately 200 cases reported) that is caused by mutations in the ATP binding cassette subfamily G members 5 or 8 (ABCG5 or ABCG8) genes,\(^1\) which result in 30–100-fold higher circulating plant sterol concentrations.\(^2,3\) The accumulation of plant sterols can manifest clinically in a variety of ways, including a lack of symptoms or death.\(^1,2\) In general, xanthomas, premature atherosclerotic cardiovascular disease, arthralgias/arthritis, and high circulating low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) concentrations are noted.\(^4,5\) In addition, more recently, anemia with stomatocytosis, macrothrombocytopenia, and splenomegaly has been identified as a typical feature.\(^6\) Instead of statins, the Niemann-Pick C1-like 1 inhibitor ezetimibe has been revealed to be the most effective drug for the treatment of this disorder.\(^5\)

However, the early diagnosis of sitosterolemia remains challenging.

Here, we report a rare case of sitosterolemia that affected multiple organ systems and occurred in a 26-year-old Chinese woman who had a novel variant in her ABCG5 gene and who had been previously misdiagnosed as having Evans syndrome and familial hypercholesterolemia (FH). This patient's case was previously reported as an example of carotid sheath xanthoma\(^7\); here we emphasize features related to the diagnosis and treatment of sitosterolemia.
Case presentation

A 26-year-old Chinese woman had a 7-year history of anemia, thrombocytopenia, and splenomegaly. She had been diagnosed with Evans syndrome and had undergone steroid therapy and splenectomy at a local hospital. However, her hematologic abnormalities did not resolve and she began to experience recurrent arthralgia, which was followed by the development of cutaneous xanthomas. In addition, her plasma TC and LDL-C concentrations remained high, despite statin treatment. Therefore, the patient was referred to our hospital for further evaluation in November 2018. The patient's parents denied consanguinity, and both her father and younger sister's blood lipids were within normal range. Although her mother had high plasma TC (5.88 mmol/L, reference range: 2.80-5.20 mmol/L) and LDL-C (4.18 mmol/L, reference range: 2.10-3.10 mmol/L), her serum lipid decreased to normal range (TC 4.76 mmol/L, LDL-C 2.94 mmol/L) after statin treatment for one month. In addition, the patient had an older brother who had died of chronic renal failure at the age of 26, but no definitive diagnosis was available.

Physical examination revealed extensive cutaneous xanthomas and extensor tendon xanthomas in areas that were subject to friction (Figure 1); asymmetrical four-limb blood pressure; vascular murmurs in regions of her neck and abdomen; and bilateral tenderness, swelling, and restriction of movement of her wrist joints. Routine hematologic findings, including her lipid profile, are presented in Figure 2. This patient presented with suspiciously positive platelet-associated IgG, negative ANA antibodies and rheumatoid factors, while a positive Coombs' test. Tenosynovitis of both wrists was demonstrated using ultrasonography,
as were multiple atherosclerotic plaques in her abdominal aorta, right common carotid artery, and left subclavian artery. Moreover, both carotid sheaths contained space-occupying hypoechogenic lesions, and their heterogeneous enhancement, which was induced using contrast-enhanced ultrasonography, suggested that they were xanthomata. However, computed tomography-angiography of her coronary and cranial arteries, and echocardiography, revealed no abnormal findings.
Figure 1. Contrast of cutaneous xanthomas on eyelids (A), extensor sites of right knee joint (C) and left elbow joint (E) on admission, and after a 21-month therapy (B, D, F and G, respectively). The size of xanthomas on bilateral eyelids decreased especially in angulus oculi medialis (B). Xanthomas on right knee joint (C) and left elbow joint (E) had been totally invisible at the last follow-up (D, F and G).
Figure 2

(A) The lipid profiles of the patient before and after the treatment with ezetimibe.

(B) The routine hematologic indices of the patient before and after the treatment with ezetimibe. The crosses indicate increased cell volume, and the five-pointed stars indicate abnormal red cell morphology such as helmet cells and target cells.
Although these findings were highly suspicious of sitosterolemia, conventional enzymatic colorimetric methods were unable to distinguish plant sterols from cholesterol, and the most up-to-date methods of qualifying plant sterol concentrations\(^5\) were not available in our hospital. Therefore, whole exome sequencing of the patient and her parents was performed, with their informed consent. Two mutations in the \textit{ABCG5} gene were found (Figure 3A), which confirmed a diagnosis of sitosterolemia. Further cascade screening identified eight heterozygous carriers in her family, but there were no other affected family members (Figure 3B).

\textbf{Figure 3}

(A)
Figure 3. (A) DNA sequence analysis of the ABCG5 gene of the patient and her parents, alongside the normal NCBI gene sequence. The arrows indicate the identified mutations. (B) Pedigree of the family. The black arrow indicates the proband (III-2). Half-filled symbols indicate heterozygous carriers: those on the mother's side of the proband carry c.1,336 (exon10) C>T, p.(R446*), while those on the father's side carry c.1,325-3(IVS9)_c.1325-2(IVS9)delCA. The symbols with an oblique line indicate the deceased relatives. The three members who are indicated by a question mark in the upper right corner did not participate in the genetic testing.

The patient was treated with ezetimibe (10 mg/day) and a low-phytosterol diet, which improved her lipid profile (Figure 2A), hematological abnormalities (Figure 2B), tenosynovitis, and arthralgia. In addition, ultrasonography revealed that her cutaneous and carotid sheath xanthomas had shrunk or resolved.

Since the assay for plant sterols is important to confirm diagnosis and assess treatment adequacy of sitosterolemia, after obtained informed consent from the patient, plant sterol analysis by high performance liquid chromatography-diode array detection (HPLC-DAD,
performed by Beijing Biotech-Pack-analytical Scientific Co., Ltd., Beijing, China) method quantified by external standard was outsourced during the follow-up period. Standard samples were all of HPLC grade (purchased from Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China). The linear equations, correlation coefficients, retention time for three standard samples and the coefficients of variation for measurements were presented in the Table 1. HPLC chromatograms of the proband and healthy controls were present in Figure 4. The proband's phytosterol concentrations were much higher than healthy controls when she stopped using ezetimibe for 10 days because she was unable to obtain ezetimibe in the local city, while rapidly reduced after reinitiating ezetimibe for only 5 days at our hospital during the follow-up (Table 2). However, the serum stigmasterol and β-sitosterol levels of three other age and weight matched healthy volunteers could not even be detected since the concentrations were below the minimum detection limit, and the serum campesterol concentration was significantly lower than that of proband (Table 2).
**Figure 4**

HPLC chromatograms of phytosterols from the standard sample, the patient off ezetimibe for 10 days (A) and on ezetimibe for 5 days (B), and three healthy volunteers (C, D and E). Peak identities are: 1. campesterol; 2. stigmasterol; 3. sitosterol. AU: absorbance unit.
Discussion

Sitosterolemia is a rare lipid storage disorder that is characterized by substantial phenotypic heterogeneity. Because of advances in detection techniques and better clinician awareness, an increasing number of cases is now being recognized. Peripheral blood smear examination is an essential part of the differential diagnosis of unexplained anemia and thrombocytopenia. In addition, ultrasonography is also a recommended screening technique for patients in which sitosterolemia is suspected. In the present case, bilateral carotid sheaths xanthomas were identified by ultrasonography, and these lesions were reduced in size by treatment with ezetimibe. Considering the high risk of hemorrhage, biopsy of carotid sheath xanthomas is not recommended, but ultrasonographic monitoring of atheromas should be performed by expert operators. Recurrent tenosynovitis is an extremely rare manifestation of sitosterolemia, having only been reported once previously, by Wadsack et al. in 2019. The symptoms of tenosynovitis were also relieved in the present patient by treatment with ezetimibe. Because timely and effective treatment can prevent disease progression, an early diagnosis of sitosterolemia is very important. The key information that is required to differentiate sitosterolemia, FH, and Evans syndrome is summarized in Table 3.

The mutation R446X (c.1,336 C>T), which is located in exon 10 of ABCG5, which was identified in five family members of this proband, is the most common gene mutation in Chinese people with sitosterolemia and has been linked to hematologic abnormalities in some patients. The other c.1,325-3(IVS9)_c.1325-2(IVS9)delCA mutation, was absent from race-matched controls in public databases (Exome Aggregation Consortium, 1000
genomes) so far. The two mutations in this case resided in trans in the ABCG5 gene demonstrated by her parents. The mutation occurs in the canonical -2 splice site, which generally could lead to loss of the gene function. According to Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology published in 2015[12], the novel splicing variant in intron 9 was pathogenic. Therefore, the genetic defects are associated with sitosterolemia. The present patient was a compound heterozygote for these two mutations, and whether the novel mutation played a significant role in her clinical phenotype has not been determined. In addition, it is noteworthy that the proband's deceased brother also had thrombocytopenia, anemia, and abnormal erythrocyte morphology. However, it is impossible to definitively determine whether he also had sitosterolemia that featured renal dysfunction and hematologic abnormalities.

Ezetimibe is currently effective in both reducing sterol concentrations and improving systemic symptoms[3], as observed in this case. Discontinuing medication without doctor's advice should be avoided. The measurement of plasma sterol levels is always recommended for making the diagnosis of sitosterolemia, and also for following the response to therapy.

Conclusions

Sitosterolemia can be a multi-system disorder and is easily misdiagnosed. Xanthoma, atherosclerosis, and hypercholesterolemia are common, non-specific manifestations of
sitosterolemia, but abnormal cellular morphology on a peripheral blood smear is relatively distinctive feature. Clinicians should include sitosterolemia in their differential diagnosis when patients that are suspected of having Evans syndrome or FH are refractory to routine therapies. Genetic analysis and the measurement of plant sterols can help confirm a diagnosis of sitosterolemia, and ezetimibe is an effective treatment.

Acknowledgments

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Ethics approval and consent to participate

The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). This case study was approved by the West China Hospital of Sichuan University Biomedical Research Ethics Committee and the consent to participate was waived. The ethics approval number is 2020-780. Written informed consent for publication of the patient’s clinical details and clinical images was obtained from the patient and her relatives.

Availability of data and materials

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.
Declarations of interest

None.

Funding

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Authors' contributions

MQ and JL diagnosed and treated the patient, reviewed the literature and wrote the main article text. PL participated in the clinical diagnosis and treatments, and helped draft the manuscript. XW contributed to ultrasound diagnosis. JL supervised this work and critically revised the manuscript. All authors have read and approved the final article.
References


Table 1. The linear equations, correlation coefficients, retention time for three standard samples and the coefficients of variation for measurements.

<table>
<thead>
<tr>
<th>Phytosterols</th>
<th>Linear equations*</th>
<th>R²</th>
<th>RT (min)</th>
<th>Intra-day</th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campesterol</td>
<td>y=8000x-21307</td>
<td>0.9998</td>
<td>44.1</td>
<td>1.5</td>
<td>2.2</td>
</tr>
<tr>
<td>Stigmasterol</td>
<td>y=20000x-28830</td>
<td>0.9994</td>
<td>46.5</td>
<td>2.7</td>
<td>1.9</td>
</tr>
<tr>
<td>β- sitosterol</td>
<td>y=9000x-55478</td>
<td>0.9988</td>
<td>53.4</td>
<td>2.6</td>
<td>2.6</td>
</tr>
</tbody>
</table>

*The x and y in the linear equations represent the peak area and concentration (mg/L) of phytosterols, respectively; R², the coefficients of determination of the standard curves; RT, retention time. The coefficients of variation of intra-day and inter-day were determined by the peak areas of the proband's samples at different storage time (0 h, 2 h, 4 h, 8 h, 12 h, 24 h) of one day and twice per day for three days, respectively.
Table 2. Plant sterols analysis by HPLC-DAD and lipid profiles of the patient and three healthy volunteers.

<table>
<thead>
<tr>
<th></th>
<th>Plant sterols (mg/L)</th>
<th>Lipid profiles (mmol/L)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Campesterol Stigmasterol β-sitosterol</td>
<td>TC LDL-C HDL-C TG</td>
<td>TC LDL-C HDL-C TG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient off EZE for 10 days</td>
<td>101.4 16.6 124.8</td>
<td>8.66 6.47 1.42 1.81</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient on EZE for 5 days</td>
<td>49.7 7.6 60.2</td>
<td>7.64 5.71 1.47 1.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy volunteer 1</td>
<td>9.5 ND ND</td>
<td>4.01 2.19 1.43 0.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Healthy volunteer 2</td>
<td>8.3 ND ND</td>
<td>3.61 1.68 1.89 0.59</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy volunteer 3</td>
<td>10.7 ND ND</td>
<td>4.30 2.29 1.82 0.85</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest limit of detection</td>
<td>3.9 3.9 7.8</td>
<td>-- -- -- --</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, total triglycerides; EZE, ezetimibe; ND, not be detected.
Table 3. Differential diagnostic methods among sitosterolemia, familial hypercholesterolemia and Evan’s syndrome.

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Familial History</th>
<th>IP</th>
<th>Major Gene Mutations</th>
<th>Clinical Manifestations</th>
<th>RBT</th>
<th>Blood</th>
<th>Sterols Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>STSL</td>
<td>Y</td>
<td>A</td>
<td>ABCG</td>
<td>√, √, √, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼</td>
<td>Di+EZ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FH</td>
<td>Y</td>
<td>A</td>
<td>LDLR</td>
<td>√, √, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼, ▼</td>
<td>E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ES</td>
<td>-</td>
<td>-</td>
<td>PCSK 9</td>
<td>- - - - - - - - - - - - - - -</td>
<td>Steroid, splenectomy</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IP, inherited pattern; GM, major gene mutations; RBT, routine blood test; FT, first-line therapy; PAS, premature atherosclerosis and correlative complications; HB, hemoglobin; PLT, platelet; ASE, abnormally shaped erythrocytes; G*, giant platelets; PS, plant sterols; TC, total cholesterol; LDL-C, low density lipid cholesterol; STSL, sitosterolemia; Y, yes; AR, autosomal recessive; ABCG5/8, ATP-binding cassette subfamily G member 5/8; DI+EZE, combined diet intervention with ezetimibe; FH, family hypercholesterolemia; AD, autosomal dominant; LDLR, low density lipid receptor; APOB,
apolipoprotein B100; PCSK9, proprotein convertase subtilisin/kexin 9; ES, Evans syndrome; ↓,#, autoimmune anemia; ↓&, autoimmune thrombocytopenia; “√”, feature can be found; “↑”, higher than the reference range; “↓”, lower than the reference range; “-”, no correlation.