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Reassessing very long chain fatty acids elevations: Sitosterolemia as a non-peroxisomal cause

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ABSTRACT

Very-long-chain fatty acids (VLCFAs) are commonly used to diagnose peroxisomal disorders, but elevated levels may also result from other non-peroxisomal causes, leading to diagnostic challenges. We report the case of a 2-year-old girl presenting with growth retardation and diarrhea, with laboratory investigations revealing elevated VLCFA levels suggestive of a peroxisomal disorder. Despite initial suspicion, genetic panels for peroxisomal and dyslipidemia-associated genes were negative. Whole exome sequencing (WES) ultimately revealed a pathogenic variant in the ABCG8 gene, consistent with a diagnosis of sitosterolemia, a rare autosomal recessive condition characterized by elevated plant sterols. Elevated plant sterols in sitosterolemia may interfere with VLCFA analysis, potentially leading to falsely elevated results and incorrect suspicion of peroxisomal dysfunction. This case underscores the importance of including sitosterolemia in the differential diagnosis for elevated VLCFA levels, particularly in patients with atypical presentations for peroxisomal disorders. It also highlights the role of WES in establishing an accurate diagnosis when biochemical findings are ambiguous. More studies are needed to evaluate the effects of plant sterols on VLCFA measurements. This report contributes to the literature by demonstrating the utility of genetic testing in clarifying challenging diagnostic scenarios involving elevated VLCFAs.

1. Introduction

Very-long-chain fatty acids (VLCFAs), defined as fatty acids with a chain length of 22 or more carbon atoms, are critical markers used in the biochemical diagnosis of peroxisomal disorders. Peroxisomal dysfunction, seen in conditions like X-linked adrenoleukodystrophy and Zellweger spectrum disorders, often presents with elevated VLCFA levels, making VLCFA analysis an essential diagnostic tool in identifying these rare metabolic conditions. However, elevated VLCFA levels do not exclusively indicate peroxisomal disorders and may result from other metabolic or dietary factors, presenting significant diagnostic challenges and risks for misdiagnosis [1].

One notable example is sitosterolemia, an autosomal recessive disorder caused by mutations in the ABCG5 or ABCG8 genes, which results in elevated levels of plant sterols, including sitosterol [2]. In affected individuals, these plant sterols may interfere with VLCFA assays, creating the appearance of elevated VLCFA levels and potentially leading to incorrect suspicions of peroxisomal dysfunction. This overlap illustrates a significant diagnostic pitfall, where misinterpreted VLCFA elevations could result in unnecessary treatments or further invasive investigations.

In this report, we present a case of elevated VLCFA levels in a patient initially suspected of having a peroxisomal disorder. The elevation of C22:0 and a slight increase in C24:0 levels raised suspicion of peroxisomal fatty acid oxidation disorders. However, normal C26:0 levels and inconsistent clinical findings highlighted the need for caution in directly associating these findings with peroxisomal dysfunction. Such parameters should be carefully interpreted in conjunction with clinical findings and supplemented by advanced diagnostic tools, such as genetic testing. Ultimately, whole exome sequencing (WES) revealed that the patient was diagnosed with sitosterolemia. This case underscores the importance of including sitosterolemia in the differential diagnosis of elevated VLCFA levels, particularly when clinical findings are ambiguous. We aim to raise awareness of sitosterolemia as a potential source of false positives in VLCFA analysis and to emphasize the role of advanced genetic testing in securing an accurate diagnosis.

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2. Case report

A 2-year-old girl was referred to the pediatric metabolism clinic due to dyslipidemia identified during following evaluations for growth retardation and diarrhea. According to her medical history, she was born at 39 weeks via cesarean section, weighing 2400 g. She had a history of being hospitalized in the neonatal intensive care unit for 5 days due to vomiting. Additionally, she received phototherapy during the neonatal period for indirect hyperbilirubinemia. Her developmental milestones were consistent with those of her peers: she sat without support at 6 months and started walking and talking at 12 months. There was a first-degree cousin marriage between her parents, and her 5-year-old sibling was healthy.

On physical examination, her weight standard deviation score (SDS) was −2.16, height SDS was −0.71, and body mass index (BMI) SDS was −3.06. There were no dysmorphic features, no organomegaly, and her neurological examination was normal.

Laboratory analysis revealed an LDL level of 201 mg/dL, C22 level of 153.13 μmol/L (normal range: 0–90.3), and C24 level of 81.74 μmol/L (normal range: 0–79.4). Systemic evaluations, including echocardiography, abdominal ultrasonography, and ophthalmologic examination, were normal. Hearing tests, which are often performed as part of the diagnostic workup for peroxisomal disorders, were conducted and revealed normal results. A genetic panel for dyslipidemia, including PCSK9, LDLR, APOE, and APOB gene analysis, yielded normal results. A peroxisomal gene panel performed for elevated VLCFA was also normal. Consequently, WES was conducted, which identified a homozygous c.1234C > T (p.Arg412*) nonsense variant in the ABCG8 (NM_022437.2) gene, classified as pathogenic. Following the diagnosis of sitosterolemia, the patient was started on a strict low-plant sterol diet, including restrictions on sterol-rich foods such as nuts, seeds, and vegetable oils. Due to the patient's young age (2 years), pharmacological treatment was not initiated. The dietary intervention alone led to significant improvements in the patient's biochemical parameters, as shown in Table 1.

3. Discussion

This case illustrates the diagnostic complexity of interpreting elevated VLCFA levels, particularly when sitosterolemia, a rare lipid disorder, is present. Although VLCFA elevations are typically associated with peroxisomal dysfunction, non-peroxisomal factors, including dietary influences and rare metabolic disorders, can lead to falsely elevated results. Sitosterolemia, in particular, exemplifies a diagnostic pitfall in VLCFA analysis: elevated plant sterols in sitosterolemia can mimic the biochemical profile of peroxisomal disorders, raising the risk of misdiagnosis and inappropriate treatment.

Table 1
Repeated Measurements of VLCFAs and Lipid Profiles Over One Year.

	Baseline	Month 3	Month 6	Month 12	Reference range
Very-Long-Chain Fatty Acids (VLCFA) (μmol/L)					
C22	**153.13**	**157.51**	**182.42**	**102.64**	0–90.3
C24	**81.74**	**86.55**	**103.34**	74.65	0–79.4
C26	0.93	**1.08**	**1.51**	0.73	0–1.008
C24/C22	0.53	0.55	0.57	0.69	0–1.3
C26/C22	0.01	0.01	0.01	0.01	0–0.026
Other Fatty Acids (μmol/L)					
Phytanic acid	N/A	3.01	3.03	1.54	0.23–5.03
Pristanic acid	N/A	0.24	0.19	0.08	<0.55
Lipid Profile (mg/dL)					
LDL	201	184	167	190	20–100
HDL	54	59	45	70	40–60
Triglyceride	315	479	247	75	20–150
Total cholesterol	292	281	233	282	40–200

Our patient's nonspecific symptoms, including failure to thrive and persistent diarrhea, prompted an investigation into potential metabolic disorders, including peroxisomal dysfunction. VLCFA measurement was thus included in the initial workup, as such findings are sensitive indicators of peroxisomal disorders. However, elevated VLCFA levels should be interpreted cautiously in cases lacking classic peroxisomal disorder symptoms, as they may result from other metabolic or dietary factors rather than intrinsic peroxisomal pathology [3].

The literature documents several non-pathological sources of VLCFA elevation, underscoring the need to contextualize VLCFA findings within each patient's dietary and metabolic background. For example, high peanut butter consumption has been linked to increased levels of VLCFAs, as shown by Lam et al. [4], who observed elevated plasma C26 levels following dietary intake of peanut products. Similarly, Theda et al. [5] demonstrated that ketogenic diets could elevate C26 levels, potentially through shifts in lipid metabolism that mimic biochemical profiles of peroxisomal disorders. Plasma sample hemolysis, another non-pathological cause, has also been reported to spuriously increase VLCFA measurements, further contributing to diagnostic confusion [1,6]. In our patient, a genetic panel for peroxisomal disorders was negative, but her VLCFA levels remained elevated, suggesting an alternative diagnosis. The increased levels of C22:0 and slightly elevated C24:0 observed in our patient may be attributed to an overall increase in plasma total lipid fatty acids, a phenomenon commonly associated with elevated plasma triglyceride levels [7]. This interpretation aligns with findings in untreated sitosterolemia, where metabolic imbalances, including elevated plant sterols, contribute to altered plasma lipid profiles [2]. These observations highlight the importance of considering broader metabolic contexts when interpreting VLCFA measurements, especially in conditions like sitosterolemia that can mimic peroxisomal disorders biochemically. Increased levels of C22:0 and slightly elevated C24:0, in the absence of raised C26:0 levels or abnormal VLCFA ratios, do not independently confirm a diagnosis of peroxisomal disorders. This underscores the importance of considering these findings within a broader diagnostic framework, including genetic testing and a detailed clinical assessment. In our case, the absence of abnormalities in the C24/22 and C26/22 ratios, along with normal C26:0 levels, guided further investigations to exclude peroxisomal disorders, leading to the eventual diagnosis of sitosterolemia. WES was essential in clarifying the diagnosis, revealing a pathogenic variant in the *ABCG8* gene consistent with sitosterolemia. This finding illustrates the utility of WES in diagnosing challenging metabolic presentations where traditional biochemical panels yield ambiguous results.

This case emphasizes the importance of a thorough nutritional history and genetic testing in patients with elevated VLCFA levels, especially when peroxisomal dysfunction is not strongly indicated by clinical presentation. Recognizing sitosterolemia as a differential diagnosis can prevent unnecessary treatment and allow for appropriate patient management, highlighting the critical role of advanced genetic testing in refining diagnostic accuracy.

Looking forward, more studies are needed to elucidate the impact of plant sterols on VLCFA measurements in sitosterolemia and other dyslipidemias. Specifically, prospective cohort studies examining VLCFA levels in individuals with known sitosterolemia could establish biochemical ranges typical of the condition, thus reducing diagnostic uncertainty. Such research would contribute to a clearer understanding of when VLCFA elevations indicate true peroxisomal dysfunction versus a benign or non-peroxisomal etiology.

In conclusion, this case underscores the diagnostic challenges of elevated VLCFA levels and the value of comprehensive approaches, including genetic testing, to differentiate between peroxisomal and non-peroxisomal causes. This report highlights the necessity of including sitosterolemia in the differential diagnosis of VLCFA elevations by contributing to the literature on this diagnostic intersection. It

demonstrates how advanced genetic testing can clarify ambiguous biochemical findings and guide accurate patient care.

Informed consent

Informed consent was obtained from the patient's family and patient in the study.

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None declared.

Contribution statement

M.Y.C. designed the study; M.Y.C., B.K., E.B and K.Y. collected and analyzed data; M.Y.C. wrote the manuscript; B.K., and EİB. gave technical support and conceptual advice. All authors read and approved the final manuscript.

Ethical approval

The local Institutional Review Board deemed the study exempt from review.

Declaration of generative AI and AI-assisted technologies

The authors declare that they did not use artificial intelligence in the writing process.

Take away message

This case underscores the necessity of considering rare causes like sitosterolemia in cases of elevated VLCFA, especially to avoid unnecessary treatment for presumed peroxisomal dysfunction.

CRediT authorship contribution statement

Merve Yoldas Celik: Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Burcu Köseci:** Supervision, Data curation. **Ezgi Burgac:** Supervision, Data curation. **Kanay Yararbas:** Data curation.

Declaration of competing interest

The authors state no conflict of interest.

Data availability

The authors do not have permission to share data.

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