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Sitosterolaemia and xanthomatosis in a child

兒童患上食物固醇血症和黃瘤病

A 4-year-old boy presented with multiple tuberous xanthomata and a fasting plasma sterol concentration of 18.3 mmol/L, consisting primarily of cholesterol. Two months after changing from an unrestricted diet to a cholesterol-lowering diet, the plasma sterol concentration decreased to 4 mmol/L. Fasting plasma cholesterol levels for his father and mother were 7.3 mmol/L and 6.0 mmol/L, respectively. The degree and rapidity of the child's response to dietary control, together with the fasting cholesterol results of both parents suggested a diagnosis of sitosterolaemia. Gas chromatography and mass spectrometry of the patient's plasma sterol levels showed that the percentage of β-sitosterol was raised at 12.76%, as was campesterol (6.26%), and stigmasterol (0.71%), confirming the diagnosis of sitosterolaemia. The addition of cholestyramine 4 g/day to a low sterol diet maintained the plasma sterol concentration at 4 to 5 mmol/L, and gradual regression of the xanthoma was observed. These findings indicate that a diagnosis of sitosterolaemia, a treatable cause of premature atherosclerosis, should be considered in children with severe hypercholesterolaemia whose plasma cholesterol level is highly responsive to dietary manipulation.

一名四歲男童呈現多塊結節狀的黃瘤，其空腹時的血漿固醇濃度為18.3 mmol/L，主要為膽固醇。經過兩個月把飲食轉為低膽固醇後，病人的血漿固醇濃度減少至4 mmol/L 。病人父母空腹時的血漿膽固醇水平分別是7.3 mmol/L 和6.0 mmol/L 。從病人對飲食控制反應的程度和速度，以及父母空腹時的膽固醇水平，可診斷病人患上食物固醇血症。此外，血漿固醇水平的氣體色譜分析和頻譜分析顯示，病人的β-食物固醇百分比升至12.76%，而菜油甾醇(6.26%)和豆固醇(0.71%)亦同時上昇，因而確定了食物固醇血症的診斷。自從把考來烯胺按每日4克的劑量加到病人的低固醇飲食後，血漿固醇濃度維持在4至5 mmol/L ，而黃瘤亦逐漸消退。這些發現顯示，對於患有嚴重的高膽固醇血症，而血漿膽固醇水平對飲食控制高度敏感的兒童，應考慮食物固醇血症的診斷；這種血症屬於一種早期的動脈硬化，而此病本身是可以治療的。

Key words:
Chromatography, gas;
Hypercholesterolemia;
Sitosterols;
Spectrum analysis, mass;
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關鍵詞：
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Introduction

Sitosterolaemia is an autosomal recessive lipid disorder characterised by the accumulation of plant sterols (mainly sitosterol), and cholesterol in the blood and body tissues. The dramatic response to dietary manipulation and bile acid binding resin therapy in sitosterolaemia differs from the response seen in familial hypercholesterolaemia, an autosomal dominant lipid disorder. Early diagnosis is essential to prevent premature atherosclerosis and to offer appropriate genetic counselling.

Case report

A 4-year-old boy presented with multiple tuberous nodules on the extensor surfaces of the ankles and elbows, the flexor surfaces of the knees, the interdigital spaces of both hands, the flexor surfaces of the metacarpophalangeal joints, and the extensor surfaces of the interphalangeal joints of the big toes. These were first noted when the child was 2 years old. Histology confirmed the nodules as xanthomata. The parents were non-consanguineous, and there was no family

Table 1. Fasting plasma lipoprotein levels obtained for the patient and his family

	Total cholesterol (mmol/L)	Triglyceride (mmol/L)	High-density lipoprotein cholesterol (mmol/L)	Low-density lipoprotein cholesterol (mmol/L)
Reference level	<5.2	<2.3	>0.9	<3.4
Patient (aged 4 years)	18.3	1.24	1.33	16.41
Mother (aged 29 years)	6.0	1.76	1.21	3.99
Father (aged 40 years)	7.3	1.81	1.39	5.09
Sister (aged 2 years)	6.4	1.24	1.93	3.91

history of xanthomatosis or premature coronary heart disease. Fasting plasma lipoprotein levels obtained for the patient, his younger sister, and his parents are shown in Table 1.

The boy's fasting plasma total cholesterol and low-density lipoprotein (LDL) cholesterol levels were significantly raised. The triglyceride and high-density lipoprotein (HDL) cholesterol levels were normal. The other family members had slightly raised total cholesterol and LDL cholesterol levels. Their fasting plasma triglyceride and HDL cholesterol levels were normal. The raised cholesterol levels observed within this family could be accounted for by a high cholesterol diet. Following dietary advice for a low cholesterol diet, the fasting plasma cholesterol levels of the father, mother, and sister were 4.5 mmol/L, 5.1 mmol/L, and 5.6 mmol/L (reference level, <5.17 mmol/L), respectively. They were all healthy and none had xanthomata.

The patient was initially treated with a low cholesterol diet and cholestyramine 1 g three times daily on the presumptive diagnosis of familial hypercholesterolaemia. His LDL cholesterol level decreased to 4 mmol/L within 2 months. In view of his parents' fasting lipid profile and the rapidity of his response to dietary treatment, sitosterolaemia was suspected.

Gas chromatography and mass spectrometry results, using reference values from the Clinical Biochemistry Unit at Queen Mary Hospital, showed that his fasting plasma sterols contained 80.27% cholesterol (reference range, 99.4%-99.8%), 12.76% β-sitosterol (reference range, 0.05%-0.15%), 6.26% campesterol (reference range, 0.01%-0.19%), and 0.71% stigmasterol (reference range, 0%-0.1%). These findings confirmed the diagnosis of sitosterolaemia. Two different mutations of the adenosine triphosphate (ATP) binding cassette subfamily G, member 5 (ABCG5 gene) were subsequently identified in this patient. The first, a missense mutation, changes the amino acid residue at position 419 from arginine to histidine, that is, R419H. The second, a novel splicing mutation, affects the invariant guanine at the first base of the donor splicing site of intron 12, that is, IVS12 + IG → A. The patient's father was heterozygous for the missense mutation, while the mother was heterozygous for the splicing mutation. No mutations were found in the patient's sister.[1]

A low sterol diet, including restricted shellfish intake, was recommended for the patient. With the addition of cholestyramine 2 g twice daily, his total cholesterol level was maintained at 4 to 5 mmol/L and the xanthomata gradually resolved. Thrombocytopenia was detected 3 years after presentation but there was no evidence of clinical bleeding.

Discussion

Sitosterolaemia was first described in two affected sisters by Bhattacharyya and Connor in 1974.[2] The condition is characterised by the presence of tendon and tuberous xanthomata, premature coronary artery disease and atherosclerotic disease, haemolytic episodes, arthralgias, and arthritis.[3-5] Mutation analysis shows a mutation in the ATP binding cassette, subfamily G, member 5 gene, or the member 8 gene on chromosome 2p21.[6]

Approximately half the patients with this condition have normal cholesterol concentrations and most of the remainder have only moderately elevated plasma cholesterol levels.[8] Two patients with high plasma cholesterol levels have been reported in the literature.[4,7] Variations in plasma cholesterol concentrations may be due to genetic heterogeneity or differences in the effects of specific dietary sterols on cholesterol metabolism and excretion.[8] Aside from the dietary explanation, the relatively high plasma cholesterol level in the patient described, along with relatively normal plasma cholesterol levels in both his parents could indicate that this patient has pseudohomozygous type 2 hypercholesterolaemia in addition to sitosterolaemia. It has been suggested that many, if not all, patients with a diagnosis of pseudohomozygous type 2 hypercholesterolaemia may have sitosterolaemia and xanthomatosis.[9]

The clinical presentation of pseudohomozygous type 2 hypercholesterolaemia is almost identical to that of sitosterolaemia, and both conditions respond well to a low cholesterol diet together with cholestyramine treatment. Two Chinese patients with pseudohomozygous type 2 hypercholesterolaemia together with sitosterolaemia have been reported in the literature.[8] Normal LDL receptor function has been reported in patients with pseudohomozygous type 2 hypercholesterolaemia.[8,10] Further studies on the binding, internalisation, and degradation of LDL in fibroblasts could help to support the additional diagnosis of pseudohomozygous type 2 hypercholesterolaemia.

The differential diagnosis for such patients also includes cerebrotendinous xanthomatosis. This condition is

characterised by xanthoma at an early age, with normal serum cholesterol levels but increased levels of cholestanol and bile alcohols. There are also neurological symptoms and signs, since the brain is involved.

There have been 45 cases of sitosterolaemia reported in the literature worldwide,[6] but the true prevalence of the disorder is not known. Sitosterolaemia is not rare locally as at least 16 cases have been confirmed by laboratory tests (personal communication). Approximately 10 patients have been reported to have thrombocytopenia as an associated feature. The exact underlying mechanism for thrombocytopenia is not known.

Plant sterols such as β-sitosterol, campesterol, and stigmasterol are structurally similar to cholesterol. Standard cholesterol assays cannot distinguish between them, so specialised tests such as gas-liquid and high-performance-liquid chromatography are necessary to confirm the diagnosis of sitosterolaemia.[7] In routine clinical laboratory testing, enzymatic colorimetric assays are most commonly used to determine the total cholesterol level in serum. These enzymes react with plant sterols as well as cholesterol, but not necessarily to the same extent. This is not a concern in routine practice since plant sterols are present only in minute amounts in human sera. However, this may create a diagnostic problem for patients with sitosterolaemia.

Plant sterol interference on standard cholesterol assays

To determine the magnitude of plant sterol interference on standard cholesterol assays, a simple experiment has been performed. Plant sterol standards were dissolved in absolute ethanol to a final concentration of 10 mmol/L and analysed for cholesterol in an automated chemistry analyser. All samples were analysed in duplicate. The TRACE Cholesterol Reagent (TRACE Scientific Ltd, Melbourne, Australia) was used, with an interassay coefficient of variation of 2.24%. The reagent to sample volume was 300:1. Three enzymatic reactions were involved:

(1) initial hydrolysis of cholesterol ester to free cholesterol using cholesterol esterase;
(2) oxidation of free cholesterol to cholest-4-en-3-one and hydrogen peroxide by cholesterol oxidase; and
(3) reaction of hydrogen peroxide with hydroxybenzoic acid and 4-aminoantipyrine in the presence of peroxidase to form a chromophore, which is quantitated at a wavelength of 500 to 550 nm.

The results are shown in Table 2.

Since the reagent to sample volume ratio is large, the matrix problem caused by absolute ethanol is expected to be negligible. The experimental results showed that different plant sterols were recognised as cholesterol by the enzymatic assay to various degrees. Thus, the total cholesterol values obtained in patients with sitosterolaemia did not reflect either the true cholesterol level in blood or the cholesterol plus plant sterol levels. In the patient in this report, both cholesterol and plant sterols contributed to the measured total cholesterol value of 18.3 mmol/L at presentation. Considering this cholesterol level, together with the clinical manifestation of widespread xanthoma, this patient may easily have been misdiagnosed as having familial hypercholesterolaemia, a better-known inherited lipid disorder. It is therefore important for clinicians to be aware of this pitfall in the standard enzymatic assay of cholesterol. Regardless of whether the cholesterol level is elevated or not, patients presenting with xanthoma, without a family history of dyslipidaemia, should be investigated for sitosterolaemia.

Table 2. Magnitude of plant sterol interference on standard cholesterol assays

Plant sterols (10 mmol/L)	Cholesterol concentration (mmol/L) obtained by standard enzymatic assay*
Cholestanol	9.1 (9.2)
β-Sitosterol	8.1 (8.1)
Stigmasterol	4.6 (4.2)
Absolute ethanol	0.1 (0.1)

* Concentration in brackets were results in duplicate

Plant sterols are plentiful in vegetable oils, nuts, whole grains, cereals and bread, fat rich vegetables, and fruits.[3] The primary metabolic defect of sitosterolaemia is believed to be enhanced intestinal absorption of cholesterol, and plant and shellfish sterols, with the secretion of these sterols from the liver into bile markedly diminished.[3,11] Xanthoma formation may be due to either the abnormal sterols directly disrupting normal intracellular sterol homeostasis or to the generalised accumulation of different sterols in all tissues causing a molecular defect in sterol metabolism.[11]

Patients with sitosterolaemia also have a reduction in total cholesterol synthesis. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase activity is significantly reduced but LDL receptor activity is normal.[3,11] Restriction of dietary intake of cholesterol, plant sterols, shellfish sterols (clams, scallops, and oysters), and the addition of cholestyramine therapy is an effective approach to treatment. 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors have no role in the management of this disorder.

Conclusion

This report documents rapid reduction of severe hypercholesterolaemia and regression of xanthomatosis in a patient with sitosterolaemia in response to dietary therapy and cholestyramine treatment. Thrombocytopenia was an associated feature in this patient.

Xanthomatosis during childhood, in the absence of a family history of premature coronary heart disease or laboratory evidence of hypercholesterolaemia in the parents, suggests sitosterolaemia rather than familial hypercholesterolaemia. Rapid and sustained lowering of serum cholesterol and regression of xanthomatosis following dietary treatment, with or without bile binding resins,

highlights the importance of early diagnosis and treatment of this condition to prevent premature coronary artery disease.

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