ABSTRACT: The liver is a key organ in lipid and lipoprotein metabolism, hence hepatic diseases often manifest as lipid disturbances. Cholestatic liver diseases are frequently associated with an important increase in total cholesterol at the expense of lipoprotein X (LpX), an abnormal lipoprotein isolated and characterized in the 1960s to 1970s in patients with obstructive jaundice. Lipoprotein X is rich in phospholipids, albumin, and free cholesterol, has a density similar to low-density lipoprotein (LDL), and a size similar to very-low-density lipoprotein (VLDL), which has hampered its detection through routine laboratory tests. Unlike LDL, LpX has no apoB-100, so it is not removed from circulation via the LDL receptor, and it is not clear whether or not it can be atherogenic. Although LpX was initially described in patients with cholestasis, it has also been found in patients with genetic deficiency of lecithin-cholesterol acyltransferase (LCAT), in patients who receive lipid-rich parenteral nutrition and most recently in patients with graft versus host disease of the liver. In the presence of LpX, plasma total cholesterol can rise up to 1000 mg/dL, which may lead to the development of skin xanthomas and hyperviscosity syndrome. Treatment of LpX-dependent hypercholesterolemia with conventional hypolipidemic drugs is frequently ineffective, and definitive treatment relies on correction of the underlying cause of cholestasis. Here, we present the case of a patient with LpX-dependent hypercholesterolemia in the context of primary biliary cholangitis.

KEYWORDS: Cholestasis, jaundice, hypercholesterolemia, lipoprotein X, apoB, graft-versus-host disease

Clinical Case

A 28-year-old woman, with a prior diagnosis of primary biliary cholangitis (PBC) 18 months before, consulted after 3 months of mild to moderate, progressive abdominal pain localized to the right hypochondrium. She reported a 2-year history of generalized pruritus with partial response to antihistamines. She also reported gradually worsening jaundice in skin and mucosae and the recent appearance of multiple white, painless, coalescing papules in her face and hands. She reported poor adherence to her medications for PBC. She denied any additional relevant medical history. On admission, the patient was in good general condition, heart rate was 78 bpm, respiratory frequency 16 bpm, blood pressure 117/69 mmHg, weight 59 kg, and height 1.61 m for a body mass index (BMI) of 22.7 kg/m². There was generalized mucocutaneous jaundice with multiple zones of post-inflammatory hyperpigmentation (Figures 1 to 3) and yellowish, well-defined papules in the perioral area (Figure 2) and interdigital folds (Figure 1). The patient had a palpable liver, 2 cm under the costal border.

Laboratory analyses showed a serum creatinine of 0.5 mg/dL, fasting blood glucose of 98 mg/dL, aspartate amino transferase (AST) of 133 UI/L (Reference value: 15–41), alanine amino transferase (ALT) of 121 UI/L (Reference value: 14–54), alkaline phosphatase of 1777 UI/L (Reference value: 32–91), total bilirubin of 9.6 mg/dL, direct bilirubin of 5.6 mg/dL, indirect bilirubin of 3.93 mg/dL, plasma albumin of 2.7 mg/dL, plasma ferritin of 560 ng/mL (Reference value: 11–307), negative serology for hepatotropic viruses and normal coagulation times. Abdominal ultrasound showed hepatomegaly (longitudinal diameter 19.6 cm) without any evidence of local or diffuse lesions in the hepatic parenchyma. The gastroenterology service started treatment with ursodeoxycholic acid 300 mg every 8 h, cholestyramine 4 g every 6 h, and oral hydroxyzine with improved itching.

At this point, a lipid panel showed a total cholesterol of 1535 mg/dL, high-density lipoprotein (HDL) cholesterol of 15 mg/dL, and triglycerides of 259 mg/dL, and the case was consulted with the endocrinology service. The initial differential diagnoses were heterozygotic familial hypercholesterolemia compounded by advanced liver disease versus a presumptive hyperlipoproteinemia secondary to lipoprotein X (LpX). Hyperviscosity complications were ruled out, and a punch biopsy of the skin lesions revealed xanthomas with extensive cholesterol deposition. Simultaneously, samples were drawn for direct low-density lipoprotein (LDL) cholesterol and plasma apolipoprotein B-100 measurement and for non-denaturing polyacrylamide lipoprotein electrophoresis. Over the first few weeks of management, the patient exhibited progressive improvement of pruritus, jaundice, and skin lesions (Figure 4), in addition to a decline in plasma markers of cholestasis (Table 1). The patient was discharged, and ambulatory management was continued with ursodeoxycholic acid, hydroxyzine, and fenofibric acid 200 mg/day.
Over the following months, the patient presented a slow but continuous improvement of the lesions in hands and face and a progressive decline in plasma cholesterol levels, although concentrations were still elevated in absolute terms (Table 1). During follow-up, the patient developed raised liver transaminases (Table 1), and fenofibrate was suspended as a preventive measure. Results from the non-denaturing agarose gel electrophoresis for the patient and 3 healthy controls were received at this point. Bands from controls exhibited a typical migration pattern with beta migration for LDL and alpha migration for HDL, whereas the patient's sample showed essentially a pattern of zero to gamma mobility, consistent with the presence of LpX (Figure 5).

Results of plasma apoB-100 measurements were completely normal for the patient's sex and age, according to reference values for the Colombian population (Table 2), confirming the diagnostic impression of LpX hypercholesterolemia secondary to PBC. Medical management of PBC was continued, and the patient was referred to a liver transplant program for definitive causal treatment.

Discussion

Regulation of plasma lipoproteins by the liver

Cholesterol is essential for multiple cellular processes, among them the maintenance of the integrity of all eukaryotic membranes. Cholesterol is also the precursor of bile acids and steroid hormones. Most endogenous cholesterol is synthesized in the liver, where the rate-limiting enzyme of cholesterol biosynthesis is 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, the therapeutic target of statins. Besides, their ability to produce cholesterol starting from acetyl-CoA, hepatocytes are also able to take up circulating LDL particles through their binding to the low-density lipoprotein receptor (LDLR) and subsequent endocytosis, thus raising intracellular cholesterol concentrations.

The production and uptake of cholesterol by liver cells are regulated through cytoplasmic concentrations. When cholesterol inside hepatocytes is high, the Sterol Response Element Binding Protein (SREBP) is bound to the SREBP-Cleavage Activating Protein (SCAP) at the endoplasmic reticulum, in an inactive conformation. When cytoplasmic cholesterol concentrations go down, SREBP is escorted by SCAP to the Golgi apparatus, where it is cleaved by the Site Proteases 1 and 2 (S1P and S2P). Once the amino-terminal portion of SREBP is released, its active carboxy-terminal fragment is translocated to the nucleus, where it acts as a transcription factor. This active SREBP binds to the promoter of the genes for the LDLRs and for HMG-CoA reductase and induces their transcription.
Greater synthesis of LDLr and HMG-CoA reductase leads to increased LDL uptake and increased cholesterol production, respectively. Both of these effects take intracellular cholesterol levels back to the normal range.

Another relevant player in liver cholesterol metabolism is the liver X receptor (LXR), a nuclear receptor activated by oxysterols, a family of compounds derived from cholesterol. When intracellular oxysterol concentrations increase, LXR activation induces the transcription of genes involved in the biosynthesis of bile acids, thus promoting cholesterol elimination. Liver X receptor activation also induces repression of the genes for squalene synthase and lanosterol 14-alpha demethylase, thus reducing de novo cholesterol production. Liver X receptor also negatively regulates the membrane transporter Niemann-Pick C1-Like 1 (NPC1L1) in enterocytes, reducing the net absorption of dietary cholesterol. Thus, the intestinal transport system for cholesterol absorption is under the control of a hepatic regulator.

The hepatic metabolism of lipids is strongly influenced by a group of nuclear receptors called the farnesoid-X receptors (FXRs). Bile acids constitute the only physiological pathway for effective cholesterol removal from the human organism. After being secreted into the duodenum and reabsorbed at the ileum, bile acids enter hepatocytes and bind to FXR, leading to repression of genes involved in bile acid biosynthesis (especially the gene for cholesterol 7 alpha-hydroxylase), and promotion of conjugation and secretion of bile acids. Thus, disrupted enterohepatic circulation of bile acids in cholestatic diseases may alter FXR-mediated feedback, leading to decreased cholesterol elimination and impaired lipoprotein homeostasis.

Table 1. Evolution of lipid profile and liver markers at admission and more than 4 months of follow-up.

|                        | ADMISSION | 1 WEEK | 4 MONTHS |
|------------------------|-----------|--------|----------|
| Aspartate amino transferase (UI/L, RV: 15-41) | 133       | 122    | -        |
| Alanine amino transferase (UI/L, RV: 14-54) | 121       | 95     | -        |
| Alkaline phosphatase (UI/L, RV: 32-91)       | 1777      | 1575   | -        |
| Total cholesterol (mg/dL)                    | 1535      | 1609   | 982      |
| HDL cholesterol (mg/dL)                      | 11        | 16     | 32       |
| Triglycerides (mg/dL)                         | 251       | 259    | 115      |
| Calculated LDL cholesterol (mg/dL)           | 1473      | 1541   | 927      |
| Directly measured LDL cholesterol (mg/dL)    | -         | -      | 499      |

A 428 mg/dL discrepancy was observed between Friedewald’s formula-calculated LDL cholesterol and directly measured LDL cholesterol at 4 months, indicating that a substantial proportion of total plasma cholesterol was located in Lipoprotein X. Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2. Plasma apoB in 3 independent replicates from the patients’ plasma, measured by ELISA.

|                     | PLASMA APOB CONCENTRATION (MG/DL) |
|---------------------|-----------------------------------|
| Replicate 1         | 55.1                              |
| Replicate 2         | 53.8                              |
| Replicate 3         | 60.8                              |

The reference value for Colombian women aged 15 to 24 is between 51.3 and 72.5 mg/dL. The presence of very high concentrations of “LDL cholesterol,” in a patient with entirely normal plasma apoB and severe cholestatic disease confirms the presence of hypercholesterolemia due to lipoprotein X. Abbreviations: ELISA, enzyme-linked immunosorbent assay; LDL, low-density lipoprotein.
expression of squalene synthase and lanosterol 14-alpha demethylase. A simultaneous increase in the expression of the membrane transporter ABCG5/G8 will lead to a net transfer of cholesterol from the intracellular compartment to circulating lipoproteins. Another relevant alteration in cholestasis is the failure to form micelle from dietary lipids and bile salts in the intestinal lumen, preventing the absorption of dietary cholesterol.

Lipoprotein X: a lipoprotein anomaly

The observation of raised plasma cholesterol in patients with intra- or extrahepatic cholestasis, or in those receiving intravenous lipid-rich nutrition solutions has been documented since several decades ago. McGinley et al described changes in the ultracentrifuge-defined lipoprotein pattern in patients with obstructive jaundice, initially interpreted as increased plasma LDL. Later, Switzer et al described a special type of "obstructive" lipoprotein, which was not recognized by antibodies raised against LDL. Seidel et al were the first to ascribe this increased plasma cholesterol to a new, uncharacterized lipoprotein they called "lipoprotein X." Our current knowledge of its pathogenesis suggests that cholestasis induces a reflux phenomenon whereby lipid fractions from bile spill over into plasma, where they combine non-covalently with albumin to conform LpX. This lack of apoB gives LpX a long half-life in circulation, as it does not contain an apolipoprotein able to bind hepatic receptors and its size prevents it from being filtered in the renal glomerulus. Therefore, LpX can only be removed from plasma by the reticuloendothelial system, mainly at the spleen. The absence of apoB also implies that LpX is unable to induce negative feedback on liver cholesterol production, and hence endogenous "regular" hypercholesterolemia may coexist with and be aggravated by LpX. The consequences of LpX accumulation encompass from skin and mucosal lesions to lipemia retinalis. In addition, the accumulation of such a large lipoprotein may cause blood hyperviscosity with associated complications, such as pulmonary embolism and/or pulmonary cholesterolomas.

Detection of LpX

LpX is similar in size to LDL and VLDL but has a different chemical composition, so it possesses a different surface electrical charge. To separate lipoproteins according to their surface charge, 1 option is to perform a non-denaturing agarose gel electrophoresis (usually at 5% agarose concentration). After running, non-denaturing agarose gels are fixed with 55% ethanol and stained with a lipophilic dye such as Sudan Black B. Given that LpX is rich in cholesterol but does not contain apoB, another way to demonstrate that extreme hypercholesterolemia in a patient with advanced liver disease is secondary to LpX is to contrast plasma total cholesterol concentrations with plasma apoB. In our patient, the exorbitant elevation of plasma cholesterol was not accompanied by increased apoB. In fact, the patient’s plasma apoB was entirely within the normal age and sex-specific reference values for the Colombian population (Table 2). A third line of evidence that reinforces a suspicion of high LpX is a high discrepancy in LDL cholesterol calculated by Friedewald’s formula (which just pools together all cholesterol that is not bound to HDL or VLDL) versus directly measured LDL cholesterol, in which chylomicrons, HDL and VLDL are precipitated with detergents and only LDL-bound cholesterol is measured (Direct LDL cholesterol kit, CAT#21585; Biosystems, Costa Brava, Barcelona, Spain). In our patient, lipid profile at 4 months showed a total cholesterol of 982 mg/dL, triglycerides of 115 mg/dL, and HDL cholesterol of 32 mg/dL, for a calculated LDL of 927 mg/dL. In the same sample, directly measured LDL cholesterol was 499 mg/dL. This difference was also strongly suggestive of a hypercholesterolemia secondary to LpX. Finally, the presence of clinical signs of extreme hypercholesterolemia confirms the laboratory diagnosis, as it happened in the case of our patient with her history of PBC, xanthelasmas, and perioral and interdigital xanthomas.

A novel method that allows the quantitative measurement of plasma LpX with an acceptable dynamic range (20-200 mg/dL) involves the separation of samples in an agarose gel, followed by staining with filipin, a dye that fluoresces when bound to free cholesterol but not when bound to neutral lipids.

Treatment of hypercholesterolemia secondary to LpX

Pharmacological therapy of LpX hypercholesterolemia differs from that of conventional polygenic or monogenic hypercholesterolemia, which relies mostly on statins, ezetimibe, and PCSK9 inhibitors. The reason is that none of these medications target the underlying pathophysiology and hence lack efficacy in this context. In the case of statins, suppression of cholesterol synthesis at the liver induces upregulation of
LDLr, but as LpX has no apoB, statins will not affect the removal of this lipoprotein by the liver. In addition, as most statins are eliminated through the bile, patients with baseline cholestatic diseases might reach toxic statin concentrations.\textsuperscript{15} In the case of ezetimibe, this agent prevents the intestinal absorption of dietary cholesterol, which has a marginal participation in the conformation of LpX. On top of that, patients with cholestasis already have a very limited absorption of dietary cholesterol due to the insufficient formation of micelle, typical of these diseases.

The use of fibrates may be considered in patients with LpX hypercholesterolemia. Although fibrates are essentially a therapy for hypertriglyceridemia, they have anti-cholestatic, anti-inflammatory, and anti-fibrotic effects in liver diseases, especially in patients with PBC. Studies of fibrate monotherapy or in combination with ursodeoxycholic acid have demonstrated significant improvements in markers of hepatocellular damage. Nonetheless, fibrate use is still not considered a first-line therapy for LpX.\textsuperscript{17}

When a patient with high LpX presents severe complications such as hyperviscosity syndrome, pulmonary embolism, or cholesterolaemia, plasmapheresis is the preferred complementary therapy.\textsuperscript{18} It should be noted though that LDL apheresis is not recommended for LpX removal, as the absence of apoB in LpX renders this therapy ineffective. Plasmapheresis has been employed not only in patients with primary liver disease but also in patients with liver graft-versus-host disease.\textsuperscript{19,20} However, its use should be considered only as a temporary measure,\textsuperscript{21} as the only definitive therapy in the case of primary liver diseases is liver transplant.

Conclusion
Hypercholesterolemia secondary to LpX is a serious and frequently neglected comorbidity of advanced liver diseases, one that does not respond to standard cholesterol-reducing drugs and can be highly disabling for the patient. It must be suspected, diagnosed, and treated opportunely to avoid life-threatening complications.

Author Contributions
LK, AG, and ED-MP were in charge of the clinical care of the patient, contributed to the writing of the case report and revised the manuscript for intellectual content. SG, KP and COM performed laboratory analyses and contributed to writing of the case report and accompanying thematic review and revised the manuscript for intellectual content. AJ contributed to writing of the case report and revised the manuscript for intellectual content.

Informed Consent
This case report was elaborated according to national and local regulations governing medical research in Colombia (Law 8430 of 1993), and in compliance with the principles of the Declaration of Helsinki. The patient herself provided written informed consent for her information and images to be published as part of this case report. Institutional review board (IRB) approval is not required for case reports deemed not to constitute research at our institution.

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REFERENCES
1. Vélez AV, Aljure JR, de Ocampo DC, de Salazar DL. Apoproteínas A1y B: valores de referencia para la población de Manizales. Acta Med Colomb. 1991;16:182-197.
2. Espenshade PJ, Hughes AL. Regulation of sterol synthesis in eukaryotes. Annu Rev Genet. 2007;41:401-427.
3. Triapant L, Segatto M, Pollortini V. Regulation and deregulation of cholesterolemia: the liver as a metabolic power station. World J Hepatol. 2012;4:184-190.
4. Xiaoping Z, Fujan Y. Regulation of SR-EfR-mediated gene expression. Sheng Wu Li Huxiu Bao. 2012;28:287-294.
5. Zhao C, Dhallman-Wright K. Liver X receptor in cholesterol metabolism. J Endocrinol. 2010;204:233-240.
6. Jia L, Batters JL, Yu L. Niemann-pick C1-like 1 (NPC1L1) protein in intestinal and hepatic cholesterol transport. Ann Rev Physiol. 2011;73:239-259.
7. Alawad AS, Levy C. FXR agonists: from bench to bedside, a guide for clinicians. Dig Dis Sci. 2016;61:3395-3404.
8. Nemes K, Aberg F, Gylling H, Isoniemi H. Cholesterol metabolism in cholestatic liver disease and liver transplantation. World J Hepatol. 2016;8:924-932.
9. Hofmann AF. Cholestatic liver disease: pathophysiology and therapeutic options. Liver. 2002;22:14-19.
10. Heinef S, Boertcher A, Kaul H, Liebsch G. Lipid profiling of lipoprotein X: implications for dyslipidemia in cholestasis. Biosci Biophis Acta. 2016;1861:681-687.
11. Seidel D, Alaupovic P, Furman R. A lipoprotein characterizing obstructive jaundice. I. Method for quantitative separation and identification of lipoproteins in jaundiced subjects. J Clin Invest. 1969;48:1211-1223.
12. Ahsan L, Ossoli A, Freeman L, et al. Role of lecithin: cholesterol acyltransferase in HDL metabolism and atherosclerosis. In: Komoda T, ed. The HDL Handbook. London: Academic Press; 2014:159-194.
13. Pelling R, Manzato A. Lipoprotein-X fifty years after its original discovery. Nutr Metab Cardiovasc Dis. 2019;29:4-8.
14. Crook MA. Lipoprotein X: clinical implications. Ann Clin Biochem. 2013;50:93-94.
15. Phathlane DV, Zemlin AE. Severe hypercholesterolemia mediated by lipoprotein X in a patient with cholestasis. Ann Hepatol. 2015;14:924-928.
16. Freeman LA, Shamburek RD, Sampson ML, et al. Plasma lipoprotein-X quantification on filipin-stained gels: monitoring recombinant LCAT treatment ex vivo. J Lipid Res. 2019;60:1050-1057.
17. Cuperus FJC, Hallbasis E, Trauner M. Fibrate treatment for primary biliary cirrhosis. Curr Opin Gastroenterol. 2014;30:279-286.
18. Wong ML, Raghavan RP, Hedger NA, Ellis RD, Meeking DR, Albon L. The use of plasmapheresis in managing primary biliary cirrhosis presenting with profound hypercholesterolemia. Br J Diabet Voc Dis. 2012;12:156-158.
19. Joukhadar R, Chiu K. Severe hypercholesterolemia in patients with graft vs host disease affecting the liver after stem cell transplantation. Endocr Prat. 2012;18:90-97.
20. Turchin A, Wiebe DA, Seely EW, Graham T, Longo W, Soiffer R. Severe hypercholesterolemia mediated by lipoprotein X in patients with chronic graft-versus-host disease of the liver. Bone Marrow Transplant. 2005;35:85-89.
21. Cohen LB, Ambinder EF, Wolke AM, Field SP, Schaffner F. Role of plasmapheresis in primary biliary cirrhosis. Gut. 1985;26:291-294.