

CASE REPORTS

Cryptogenetic liver cirrhosis and prothrombotic mutations - A mere association?

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ABSTRACT

Thrombin activation and microthrombosis of intrahepatic portal venules is a common feature in liver cirrhosis, due in part to relative protein C deficiency and altered coagulation-anticoagulation-fibrinolysis balance. Extension of this microthrombotic process to larger portal vessels explains the increased incidence of portal vein thrombosis in liver cirrhosis. Thrombin not only leads to thrombosis, but also activates liver stellate cells and promotes fibrogenesis. Also, ischemia associated with thrombosis up-regulates the expression and secretion of growth factors involved in fibrogenesis. The coincidence in a given patient of prothrombotic mutations, such as factor V Leiden or PAI-1 polymorphisms, can accelerate the fibrogenetic process. We hereby present two cases of liver cirrhosis in which etiologic evaluation was negative except for the finding of a factor V Leiden mutation in one case and the 4G/5G PAI polymorphism in the second case. These observations support the hypothesis that these mutations may be involved in the etiology of some cases of cirrhosis, or, at least, accelerate the evolution of the disease. It is therefore convenient to search for the presence of prothrombotic mutations in patients with cryptogenetic cirrhosis.

Key words: Cirrhosis. Factor V Leiden. Portal vein thrombosis. Protein C. Prothrombotic mutations. Fibrogenesis.

INTRODUCTION

The importance of microthrombosis of portal venules in liver cirrhosis has been recently highlighted. In cirrhotic patients, thrombosis of major branches of the portal vein is frequently observed, partly due to venous stasis, and possibly, to endotoxemia-mediated endothelial damage (1). Both liver ischemia and endothelial fibrin deposition may trigger progressive fibrogenesis. In this sense, a recent study suggests that prophylactic anticoagulation with enoxaparin leads to a delay in the derangement of liver function over time (2). Thrombin activates transforming growth factor (TGF)- β , and ischaemia up-regulates synthesis of hypoxia inducible factor 1 α (HIF-1 α), which in

turn promotes secretion of platelet derived growth factor (PDGF) and other growth factors, all of them potent activators of hepatic stellate cells (3).

Mutations of factors involved in coagulation and/or fibrinolytic pathways have been described, such as factor V Leiden, prothrombin, or plasminogen activator inhibitor (PAI-1) mutations, all of them leading to a prothrombotic state. It has been observed that the presence of some of these mutations in cirrhotic patients leads to an accelerated progression of the disease. Such an effect has been reported in patients with factor V Leiden (4), and in carriers of the G20210A prothrombin mutation (5). Moreover, it has been speculated that these prothrombotic alterations may even play an etiologic role in liver cirrhosis (6). However, as said, this remains speculative. Carefully conducted case control studies as well as experimental research with mutant murine models are required to confirm this hypothesis.

We hereby present two patients with cryptogenetic cirrhosis where the only identifiable underlying alterations were factor V Leiden mutation in one of them and PAI-1 mutation in the other. These cases support the hypothesis previously mentioned.

CASE REPORTS

Case 1

A sixty four year-old woman, who denied any alcohol intake, was admitted for abdominal distention and swollen legs. A diagnosis of cryptogenetic liver cirrhosis had been previously established, based on clinical grounds and liver ultrasound. She remained in her normal status until about 4 months before admission, when she began with progressive weakness, weight loss, swollen legs and an increased abdominal circumference. Physical examination showed

Received: 02/06/2015
Accepted: 14/07/2015

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Monereo-Muñoz M, Aguilera-García SG, De-la-Barreda-Heusser R, González-Arnay E, García-Hernández S, González-Reimers E. Cryptogenetic liver cirrhosis and prothrombotic mutations - A mere association?. *Rev Esp Enferm Dig* 2016;108(9):588-591.

DOI: 10.17235/reed.2015.3871/2015

moderate to severe malnutrition, petechiae, hepatomegaly, splenomegaly, ascites, collateral abdominal circulation and a right pleural effusion. Laboratory evaluation was notable for thrombocytopenia ($86 \times 10^9/L$), near-normal prothrombin activity (84%) mild hypoalbuminemia (36 g/L) and hyperbilirubinemia (1.9 mg/dL), and slight cholestasis, with raised gamma glutamyl transpeptidase (GGT 195 U/L; normal = 7-40 U/L) and alkaline phosphatase (279 U/L, normal 35-104 U/L); aspartate and alanine aminotransferase (ASAT and ALAT) as well as glycated hemoglobin levels were in the normal range. C reactive protein (CRP) levels were also raised (17.90 mg/L). Other laboratory tests searching for usual secondary causes of cirrhosis were all negative, including antinuclear, anti-liver kidney microsomal fraction, antimitochondrial and anti smooth muscle antibodies; serology against hepatitis B, C and E, iron, ferritin, ceruloplasmin and copper, and $\alpha 1$ antitrypsin.

Abdominal ultrasound showed large ascites, collateral circulation, splenomegaly and a heterogeneous liver with cirrhotic appearance. Peritoneal fluid analysis was consistent with a non-infected, non-neoplastic transudate. The patient was placed on diuretics (spironolactone + furosemide). A few days after admission, the patient complained of a mild epigastric postprandial discomfort. A computed tomography scan (CT) revealed thrombosis of the portal, superior mesenteric and splenic veins, without any data of malignancy. A transjugular liver biopsy was performed, confirming the diagnosis of (non-specific) cirrhosis. In order to study the nature of the thrombotic event we searched for antiphospholipid antibodies, which were negative, and performed a genetic study searching for prothrombotic mutations. The patient showed normal genotype for prothrombin and PAI-1 promoter, but she was heterozygote for factor V Leiden. Serum α -fetoprotein was normal.

Low-molecular weight heparin was added to the treatment, but the patient suffered a protracted course, with ascites partially refractory to diuretics, development of acute renal failure (creatinine levels reached 2 mg/dL), pleural effusion, and later, disorientation, confusion and flapping tremor consistent with hepatic encephalopathy, dying 2 months after admission.

Case 2

A 65-year-old woman with a history of dyslipemia and diabetes (for which she was placed on metformin) was admitted to the hospital due to a urinary tract infection caused by *Escherichia coli*. She denied any alcohol intake, but she reported a weight loss of about 15 kg in the last 2 months that she attributed to reduced appetite. Physical examination at admission showed splenomegaly and a non tender 10 cm hepatomegaly, findings that were confirmed by ultrasound examination. Laboratory evaluation

at admission showed hemoglobin 11.9 g/dL, leukocyte count ($13 \times 10^9/L$), prothrombin activity 72%, ASAT 43 U/L, bilirubin 1.3 mg/dL and CRP 63.7 mg/L. Regarding the study of the etiology of liver cirrhosis, we found negative HCV, HBV, and HEV serology as well as negative antinuclear, anti-liver kidney microsomal fraction, antimitochondrial and anti smooth muscle antibodies. No data of portal hypertension were observed on esophagogastroscopy, so a diagnosis of cryptogenetic or, possibly, autoimmune cirrhosis was established and the patient was placed on prednisone. Two months later she was admitted again due to a second urinary tract infection. New data on physical examination included petechiae on abdominal wall and proximal thighs. There was a worsening of liver function with prothrombin activity that dropped to 57%, hemoglobin of 11.5 g/dL and platelet count of $61 \times 10^9/L$. Hemochromatosis, α -1 antitrypsin deficiency and Wilson disease were also excluded during this admission (normal values of iron, ferritin, ceruloplasmin copper and α -1 antitrypsin), and a liver biopsy was performed in which fibrous tracts surrounding regeneration nodules were observed with proliferative changes in biliary ducts, moderate lymphocytic infiltration and moderately intense inflammatory activity. Azathioprine was added to the treatment, and the patient was discharged. She was admitted in four further occasions due to urinary tract infections. A cystoscopy was performed, without any alteration. During these admissions liver function remained stable (prothrombin activity 95%), but anemia (hemoglobin, 9.1 g/dL) and hypoalbuminemia (2.8 g/dL) ensued, possibly in association with recurrent urinary infections. Also, dissociated cholestasis was detected (alkaline phosphatase 230 U/L; GGT 176 U/L). Given the uncertain etiology of liver cirrhosis we performed a genetic study searching for prothrombotic mutations. The patient showed normal genotype for prothrombin and factor V but she showed a 4G/5G PAI-1 polymorphism. Immunosuppressive agents were discontinued and she has been followed up as outpatient, having shown a favorable outcome until now.

DISCUSSION

We present the cases of two women of similar age, affected by biopsy-proven liver cirrhosis. None of them were alcohol consumers and the search for usual secondary etiologies was negative, but both showed prothrombotic mutations: factor V Leiden in the first case and PAI-1 4G/5G polymorphism in the second one. Strikingly, both patients also showed abdominal petechiae and dissociated cholestasis in laboratory evaluation.

As previously commented, in liver cirrhosis excessive activation of thrombin is commonly found (7), due in part to endothelium damage of portal venules with tissue factor liberation (8), relative deficiency of C protein (9) and factor VIII excess (10). The procoagulant effect of thrombin not

only leads to local portal vein thrombosis, but also induces activation of fibrogenic factors such as TGF- β (6). In addition, local thrombosis of portal sinusoids produces ischemia that activates HIF-1 α (11) and leads to increased expressions of other profibrogenic factors, including the most powerful of them, PDGF (12). This explains the so called parenchymal extinction phenomenon, an ischemic process in which necrotic tissue is substituted with fibrosis. Therefore, thrombin activation has two consequences on progressive fibrous tissue deposition: a direct effect, mediated by TGF- β activation, and an indirect one related to several growth factors ultimately derived from ischemia secondary to thrombosis of portal venules.

All these facts derived from thrombin activation support the hypothesis that the mere activation of coagulation may promote cirrhosis progression. Logically, the presence of prothrombotic conditions may aggravate and/or accelerate this process. Factor V Leiden prevents the normal anti-thrombin activity of activated protein C. Regarding the second patient, PAI-1 4G/5G polymorphism has been associated with an increased thrombotic risk (specifically, to a 6-fold increase in portal vein thrombosis, 13). Moreover, PAI-1 exerts a profibrogenic effect, inhibiting plasmin dependent metalloproteinase (14), although it is not clear if this effect is greater in carriers of the 4G/5G polymorphism.

Some studies have shown that fibrosis progression is faster in patients with hepatitis C virus infection who also show factor V Leiden or are carriers of other prothrombotic polymorphism (15). Also, Villa et al. (2) clearly showed that prophylactic enoxaparin improved evolution of cirrhosis compared with placebo. The observations reported in this study suggest that it is possible that the presence of prothrombotic mutations may lead to liver cirrhosis, or at least, contribute to an accelerated progression of already established (cryptogenetic) cirrhosis, as it may be the case of the first patient reported.

To conclude, cryptogenetic liver cirrhosis is a clearly defined entity. However, some factors can influence the progression of the disease, such as prothrombotic polymorphisms of PAI-1, prothrombin, and factor V Leiden. Despite the relative paucity of studies in this field, the

cases here reported support the importance of searching for prothrombotic mutations in the etiologic evaluation of cryptogenetic liver cirrhosis.

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