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Liver infection and COVID-19: the electron microscopy proof and revision of the literature

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Abstract. – OBJECTIVE: COVID-19, the newly emerging infectious disease, has been associated with acute liver injury, often related to progression to severe pneumonia. The association between moderate-severe liver injury and more severe clinical course of COVID-19 has suggested that liver injury is prevalent in severe than in mild cases of COVID-19, while no difference in liver involvement has been reported between survivors and non-survivors. The spectrum of liver involvement during COVID-19 ranges from an asymptomatic elevation of liver enzymes to severe hepatitis. Only rarely, cases with acute hepatitis have been reported in the absence of respiratory symptoms. Both epithelial and biliary cells possess the angiotensin-converting enzyme-2 receptors that SARS-CoV-2 uses to be internalized. However, to our knowledge, no ultrastructural identification of the virus in liver cells has been reported to date. Here we provide evidence of SARS-CoV-2 in the liver of two patients, a 34-year-old woman and a 60-year-old man with COVID-19.

PATIENTS AND METHODS: We investigated two patients with COVID-19 showing several virions within cytoplasmic vacuoles of cholangiocytes and in endothelial cells of hepatic sinusoids. In both patients, we performed histological and ultrastructural examinations by liver biopsy. After two months, both patients were free of symptoms, and the SARS-CoV-2 infection had resolved.

RESULTS: Liver biopsy histological and ultrastructural examination showed liver injury and several virions within cytoplasmic vacuoles of cholangiocytes and in endothelial cells of hepatic sinusoids.

CONCLUSIONS: Although most studies in COVID-19 have been focused on the lungs, recently, cholestatic liver pathology has been introduced in the spectrum of pathological changes related to COVID-19. To the best of our knowledge, those presented in this paper are the first images of hepatic SARS-CoV-2 infected liver cells. Our findings suggest a role for cholangiocytes and biliary structures in the COVID-19.

Key Words:
SARS-CoV-2, COVID-19, Liver disease.

Introduction

Coronavirus disease 2019 (COVID-19) is a newly emerging infection disease, declared as a global emergency by the World Health Organisation[1].

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In multiple reports worldwide, COVID-19 has been associated with acute liver injury (ALI) manifested by increased serum liver enzymes[2]. Cai et al[3] focused on liver involvement in patients with COVID-19 evidenced abnormal liver function tests in 3 out of 4 patients, whereas liver injury was diagnosed in more than 20% of hospitalized patients, often associated with progression to severe pneumonia. Phipps et al[4] carried out in a large cohort of patients affected by COVID-19 and focused on the prevalence of acute liver injury in carriers of SARS-CoV-2 revealed mild liver injury in 45%, moderate in 21%, and severe liver injury in 6.4%. The latter showed alanine aminotransferase (ALT) serum levels > 5 times the upper limit of normal, and the majority showed a more severe disease course. Patients with severe COVID-19, undergoing acute respiratory distress syndrome (ARDS), were reported to show higher rates of liver injury, when compared with subjects with less severe disease[5]. The association between moderate-severe liver injury and a more severe clinical course of COVID-19 was indicated, suggesting that liver injury is more prevalent in severe than in mild cases of COVID-19[6]. As for the prognostic value of liver involvement in COVID-19 patients, no difference has been reported between survivors and non-survivors[7].

The spectrum of liver involvement during COVID-19 ranges from an asymptomatic elevation of liver enzymes to severe hepatitis[8,4]. Epithelial and biliary cells possess the angiotensin-converting enzyme-2 receptors that severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2) uses to bind to cells and to be internalized[9]. Only rarely, cases with acute hepatitis have been reported in the absence of respiratory symptoms. In one of these patients, transaminase serum levels were detected very high (> 20 times normal values), but abnormalities in liver function tests quickly normalized[10].

However, to our knowledge, no ultrastructural identification of the virus in liver cells has been reported to date. Here we provide evidence of SARS-CoV-2 virions in the liver of two patients who presented during the COVID-19 pandemic with signs and symptoms of severe intrahepatic cholestasis and underwent a liver biopsy.

Patients and Methods

Electron Microscopy

Electron microscopy examination was performed on the two liver biopsies fixed in glutaraldehyde solution in order to search for the presence of virus particles suggestive of SARS-CoV-2 infection.

Genetic Studies

Blood samples of the two patients with SARS-CoV-2 infection and cholestasis were tested for genetic causes of intrahepatic cholestasis. Whole exome sequencing was performed focusing on a mutational analysis on the 3 main intrahepatic cholestasis genes (ATP8B1, ABCB11, and ABCB4) and 66 genes causative of genetic syndromes with intrahepatic cholestasis as one of the clinical signs.

Results

Case 1

A 34-year-old woman was evaluated for abdominal pain, nausea, jaundice, and pruritus. Due to the ongoing COVID-19 pandemic, a nasopharyngeal swab was performed, which turned positive for SARS-CoV-2 infection; so, the patient was admitted to a dedicated unit. Abdominal ultrasound and magnetic resonance cholangiopancreatography (MRCP) excluded biliary duct dilation and signs of chronic liver disease. Nevertheless, two endoscopic cholangiopancreatographies were performed, and a small stone was removed from the extrahepatic biliary duct. Due to worsening cholestasis, a decision was thus made to obtain a percutaneous liver biopsy. Histological examination showed mild bilirubin accumulation within biliary canaliculi and in the cytoplasm of Kupffer cells. Neutrophils were seen in sinusoids and scanty lymphocytes in portal spaces. Electron microscopy findings are shown in Figure 1, Panels A-D. The patient tested negative for the 3 main intrahepatic cholestasis genes, while was heterozygous for a known pathogenetic mutation (c.964-1G>C) in the DHCR7 (dehydrocholesterol reductase) gene responsible for Lemli-Opitz syndrome (MIM 270400).

Case 2

A sixty-year-old man became severely jaundiced and symptomatic for pruritus two weeks after having suspended the use of a gym supplement (LGD-4033; Ligandrol®; Ligand Pharmaceuticals Incorporated, San Diego, CA, USA) capsules, that he had been consuming in the pre-

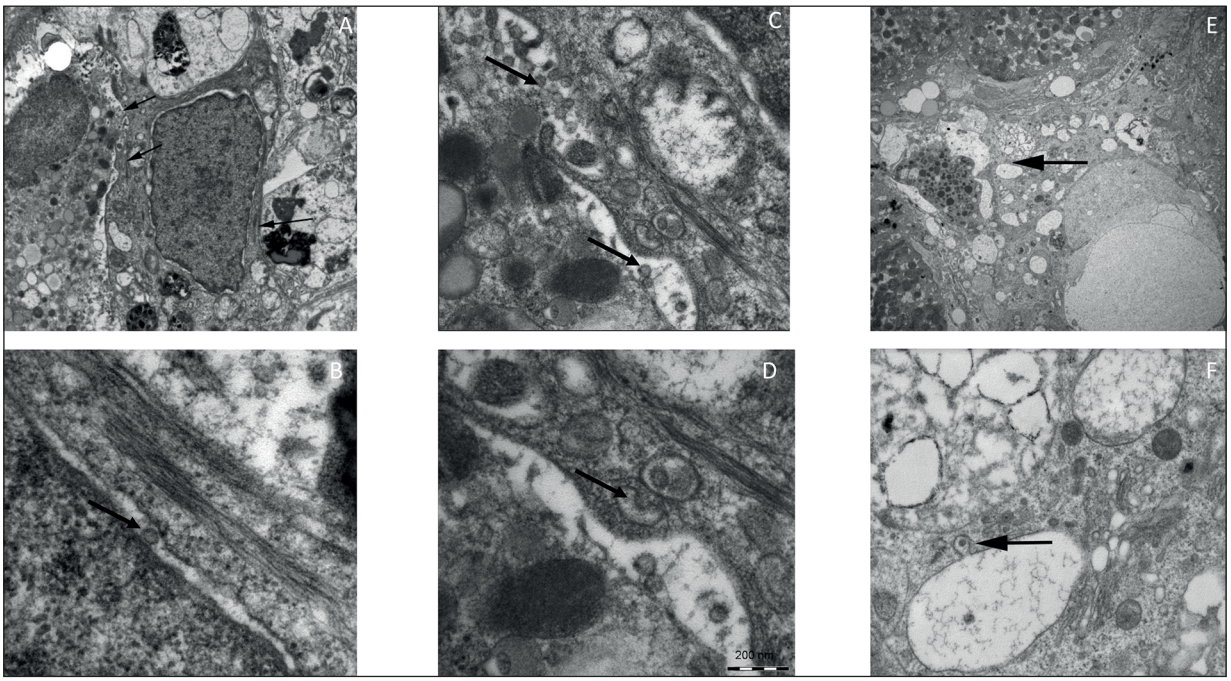

Figure 1. Electron microscopy evidence of SARS-CoV-2 infection in liver cells. **Case 1, A-D.** Cholangiocyte showing several virions within cytoplasmic vacuoles, lining the plasmalemma and within the biliary lumen (*arrows*). Virus-like particles were also observed within the perinuclear cisternae (*arrow*, **B**). The mature virions, about 60-85 nm in diameter, were composed of an inner and an outer part. The inner part was usually electron-lucent with variable amounts of peripheral granular electron-dense material, corresponding to the sections of the nucleocapsid. The outer part consisted of a lipidic envelope with club-like structures or "spikes" about 15-20 nm long. Note an image of budding (*arrow*, **D**). Original magnification: **A,** 7000×; **B, D,** 85000×; **C,** 50000×. **Case 2, E-F.** A virus-like particle of about 80 nm, with spikes, within a cisterna of rough endoplasmic reticulum, in an endothelial cell of hepatic sinusoids. Original magnification: **E,** 3000×; **F,** 20000×.

ceding four weeks. He had stopped taking the capsules because he had noticed that the urine was strongly colored. A MRCP did not demonstrate abnormalities of the biliary tree. A decision was made to admit him as an in-patient for a percutaneous liver biopsy while whole exome sequencing results were pending; due to the cautionary rules adopted in the hospital during the ongoing COVID-19 pandemic, he underwent two nasopharyngeal swabs, one of which turned positive for SARS-CoV-2 infection. Liver biopsy showed bile plugs in dilated canaliculi, mostly in perivenular location, without cholestatic rosettes and only mild infiltration of neutrophilic and lymphocytes in sinusoids and portal spaces. Electron microscopy findings are shown in Figure 1, Panels E-F. Genetic studies showed that the patient carried a heterozygous pathogenetic mutation (p.Ser320Phe) in the ATP binding cassette subfamily B member 4 (ABCB4) gene, previously described in compound heterozygous patients with progressive familial intrahepatic cholestasis, in heterozygous patients with intrahepatic cholestasis of pregnancy (MIM 614972), drug-induced cholestasis, low phospholipid-associated cholelithiasis (MIM 600803).

Course

The course of liver biochemistry for both cases is presented in Table I. Two months after the liver biopsy, both patients were free of symptoms of cholestasis, and Sars-CoV-2 infection had resolved.

Discussion

Although most studies in COVID-19 have been focused on the lungs, recently, liver pathology has been introduced in the spectrum of pathological changes related to COVID-19[2]. The histological liver changes appear unspecific, mainly characterized by microvesicular steatosis[11]. Previous electron microscopic studies evidenced the presence of SARS-CoV-2 in human airway epithelial cells[12]. SARS-CoV-2 particles were detected in

Table I. Time course of liver biochemistry tests.

	At presentation	At the time of liver biopsy	At last follow-up visit
Case 1			
AST, U/l	43	80	60
ALT, U/l	102	148	82
ALP, U/l	441	391	417
GGT, U/l	68	18	23
Bilirubin, mg/dl			
Total	6.2	15.6	2.7
Direct	5.2	13.0	2.1
Case 2			
AST, U/l	90	64	44
ALT, U/l	254	75	44
ALP, U/l	NA	440	277
GGT, U/l	24	23	24
Bilirubin, mg/dl			
Total	17.0	11.3	3.2
Direct	12.7	9.0	2.6

ALT (alanine aminotransferase): normal range 0-40 U/l; AST (aspartate aminotransferase): normal range 0-40 U/l; ALP (alkaline phosphatase): normal range 70-290 U/l (female), 90-360 U/l (male); GGT (gamma-glutamyl transferase): normal range 0-50 U/l. Total bilirubin: 0.3-1.2 mg/dl.

lung tissue by transmission electron microscopy, appearing enclosed in single-membrane vacuoles[13]. Coronavirus-like particles were detected in the respiratory system, kidney and gastrointestinal tract[14]. Electron microscopy of kidney biopsies from a patient affected by COVID-19 showed the presence of viral inclusion bodies in endothelial cells. Viral particles were observed in peritubular spaces of endothelial cells of the glomerular capillaries. Aggregates of viral particles were characterized by a dense circular surface and a lucid center[15]. The size of coronaviruses has been reported to range between about 80 and 140 nm[16]. Accordingly with what was observed in a SARS patient by Goldsmith et al[17] in 2004, virions showed spherical shape, 78 nm in mean diameter, and were composed of a helical nucleocapsid within an envelope with surface projections.

To the best of our knowledge, those presented in this paper are the first images of hepatic SARS-CoV-2 infected cells, detecting that the liver is a target for this virus. Moreover, the present report adds to the fast-growing literature on COVID-19 the suggestion that SARS-CoV-2 infection, temporally related to intrahepatic cholestasis, may occasionally favor its development in the presence of other cofactors. Gene variants of ABCB4, coding for a glycoprotein also known as multidrug resistance protein 3, have indeed been associated with the full spectrum of cholestatic diseases, with low, normal or high glutamyltranspeptidase[18]. Regarding the DHCR7 mutation detected in Case 1, the mutation impairs the DHCR7 protein expression severely, but no clinical manifestations in heterozygous carries have been reported yet[19]. Therefore, in our cases, biliary lithiasis may have played a role.

Conclusions

Based on recent literature, COVID-19 appears a complex systemic disease triggering multiple molecular pathways in different organs[20]. Multiple recent works have highlighted the putative role of liver involvement in the physiopathology and outcome of infection by SARS-CoV-2, suggesting a peculiar "hepatic tropism" for this virus[21]. The two described cases confirm the hypothesis of a putative central role of the liver in the pathophysiology of COVID-19. The finding of viral structures in cholangiocytes is the first evidence of SARS-CoV-2 cholangiocellular infection so far reported in COVID-19 patients. Our outcomes confirm previous data in human liver ductal organoid cultures in which, following SARS-CoV-2 infection, cholangiocytes underwent pathological changes[22]. Moreover, our results reinforce the significance of the one case report of SARS-CoV-2 RNA in bile[23] and confirm the hypothesis that the gut-liver-bile-gut axis might represent a vicious circle which might increase the chances of survival for the virus[21].

Many crucial questions remain open regarding the role of liver involvement in COVID-19 patients. Our findings suggest a role for cholangiocytes and biliary structures in this disease. Future research on the role of the liver in COVID-19 is needed to set up more appropriate therapeutic and preventive programs regarding liver involvement. In particular, patients like those here reported, with cholestatic features and with signs of damage of the biliary epithelium, should undergo long-term hepatic follow-up in order to prevent the insurgence of secondary sclerosing cholangitis (SSC) previously described in critically ill patients (CIP)[24].

Conflict of Interest

The Authors declare that they have no conflict of interests.

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