Cholangiocarcinoma with a Background of Hepatitis B Virus-associated Cirrhosis

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Reprinted from Internal Medicine
Vol. 40, No. 5, Pages 382–385
May 2001

□ CASE REPORT □

Cholangiocarcinoma with a Background of Hepatitis B Virus-associated Cirrhosis

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Abstract

Recently, hepatitis virus-associated chronic hepatitis or cirrhosis has been suggested to be involved in the pathogenesis of cholangiocarcinoma (CC). A 52-year-old man was diagnosed as CC with a background of hepatitis B virus (HBV)-dependent cirrhosis. A minute hepatic tumor was found during the follow-up, and was diagnosed as CC on percutaneous biopsy. The patient died of hepatic failure and an autopsy revealed the tumor to be a well to moderately differentiated adenocarcinoma. An immunohistological analysis of HBV X gene-encoded protein (HBX) was neither detected in the cancerous nor in the noncancerous tissue. No oncogenic role of the virus was verified in this case. (Internal Medicine 40: 382–385, 2001)

Key words: liver cirrhosis, hepatitis B virus, viral protein, immunohistochemistry

Introduction

Intrahepatic choangiocarcinoma (CC) is a malignant neoplasm arising from the intrahepatic biliary epithelium. Although the precursor lesions are not identifiable in the majority of CC cases, several hepatobiliary diseases, including hepatolithiasis, primary sclerosing cholangitis, liver fluke infestation, Caroli's disease, and longstanding Thorotrast deposition, are known to be predisposing factors (1–3).

Hepatocellular carcinoma (HCC) is frequently associated with liver cirrhosis, for example in 58–78% of cases in Japan (4–6). While only 1.8–30% of CC occur in cirrhotic livers (3–8), recently, hepatitis virus-associated chronic hepatitis or cirrhosis have been suggested to be involved in the pathogenesis of this tumor (9, 10). As patients with chronic hepatitis or cirrhosis are usually followed-up closely for the detection of HCC by ultrasonography, minute CC could be detected (10–12). In Japan, 23.1–32% of CCs have been found in individuals with anti-hepatitis C virus (HCV) antibodies in their sera (5, 6, 10), being frequently associated with cirrhosis (9, 10). In contrast, only 6.9–10% of CCs were found in hepatitis B virus (HBV) surface antigen (HBsAg)-carriers (3, 4–6, 10), and links between CC and HBV-associated cirrhosis have only rarely been reported (7, 9, 11–13). An etiological relationship has not been established, however, HBV X gene-encoded protein (HBX), which has been shown to transactivate viral and cellular genesis, including transcriptional factors and growth-regulating genes (14), and to induce neoplastic transformation of the infected cells (14), was studied immunohistochemically in CC patients with hepatitis B virus-associated chronic hepatitis (15). We report a case of CC which occurred in a cirrhotic liver expressing viral proteins of HBV. HBX was not demonstrated in the tumor immunohistochemically and no oncogenic role of the virus was verified in this case.

Case Report

A 52-year-old man visited our clinic on May 10, 1996, suffering from abdominal fullness. He had no family history of liver diseases and no past history of alcohol intake or blood transfusion. In 1986, he developed chronic hepatitis B, and received interferon therapy several times in another institution. Physical examination revealed massive ascites and edema in his lower extremities. Laboratory data were as follows; total bilirubin, 1.9 mg/dl; aspartate aminotransferase (AST), 144 IU/*l*; alanine aminotransferase (ALT), 149 IU/*l*; alkaline phosphatase, 229 IU/*l* (normal <223); lactate dehydrogenase (LDH), 355 IU/*l*; 15-minute indocyanine green, 32%; ammonia, 89 μg/ml (normal <70); HBsAg+; HBsAb–; HBeAg–; HBeAb+; HBcAb+; HBV-DNA, 24 pg/ml (Hybridization method); HCV-(3rd generation, Chiron, USA); α-fetoprotein (AFP), 305 ng/ml (normal <20); white blood cells, 3,890/mm^3; red blood cells, 417x10^4/mm^3; hemoglobin, 13.8 g/dl; platelet, 3.1×10^4/mm^3, its prothrombin time, 75.2%. Ascites resulted from a transudate. The diagnosis was HBV-associated liver cirrhosis with

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Received for publication June 6, 2000; Accepted for publication December 28, 2000

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hepatic failure. A hypoechoic tumor (2×2 cm) in the hepatic hilar region was detected by ultrasound in May 1997. On magnetic resonance imaging (MRI) the tumor appeared as a small mass with clear borders, hypointense on a T1-weighted image, isointense on a T2-weighted image and hypointense with a dense ring enhancement in the early phase after bolus injection of 0.05 mmol/kg gadopentetate dimeglumine (Fig. 1). Levels of tumor markers were as follows; AFP, 115 ng/ml; carcinoembrionic antigen, 16.9 ng/ml (normal <2.5); CA19–9, 64 U/ml (normal <37). Hepatic angiography showed no significant findings. In October 1977, the tumor was diagnosed as a CC on examination of a needle biopsy. Because of hepatic failure, resection of the tumor was not recommended. In April 1998, jaundice appeared, and MRI study revealed an enlargement of the tumor. Carcinoembrionic antigen was 10.9 ng/ml, and CA 19–9 rose to 1,013 U/ml, while AFP was within the normal range. In July 1998, the patient died of hepatic failure.

Autopsy revealed that the main tumor (CC), 6×6×4 cm in size, was localized in the hepatic hilar region. Histologically, the tumor was a well to moderately differentiated adenocarcinoma (Fig. 2). Several small satellite nodules were observed surrounding the main tumor and infiltration along the intrahepatic bile ducts was evident, with small metastatic nodules noted in the gall bladder wall. There was no metastasis in other organs. Expression of CA 19–9, but not AFP, in the tumor tissue was evaluated as demonstrated by the avidin-biotin-peroxidase

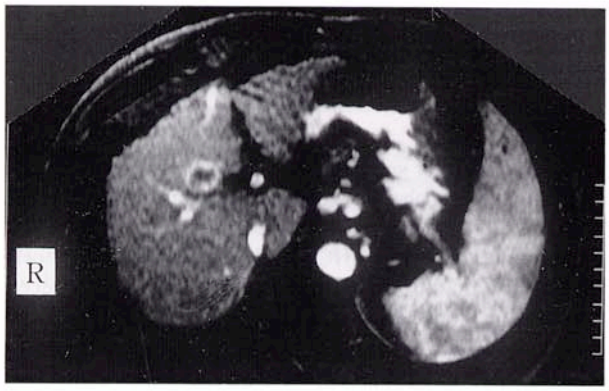


Figure 1. Dynamic MRI shows hypointense mass with a dense ring enhancement in the early phase after bolus injection of 0.05 mmol//kg gadopentetate dimeglumine (Gd-DTPA) in the hilar region.

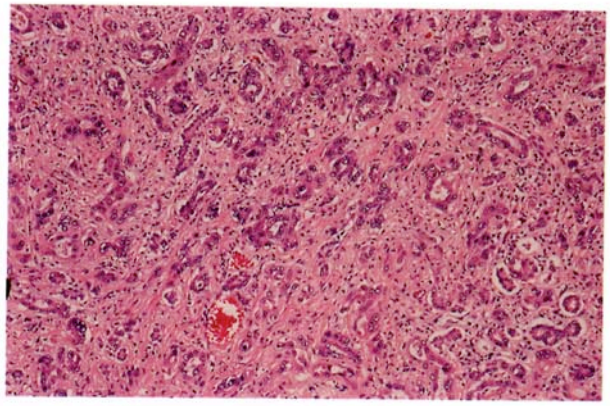


Figure 2. Well to moderately differentiated hilar cholangiocarcinoma (HE stain, ×400).

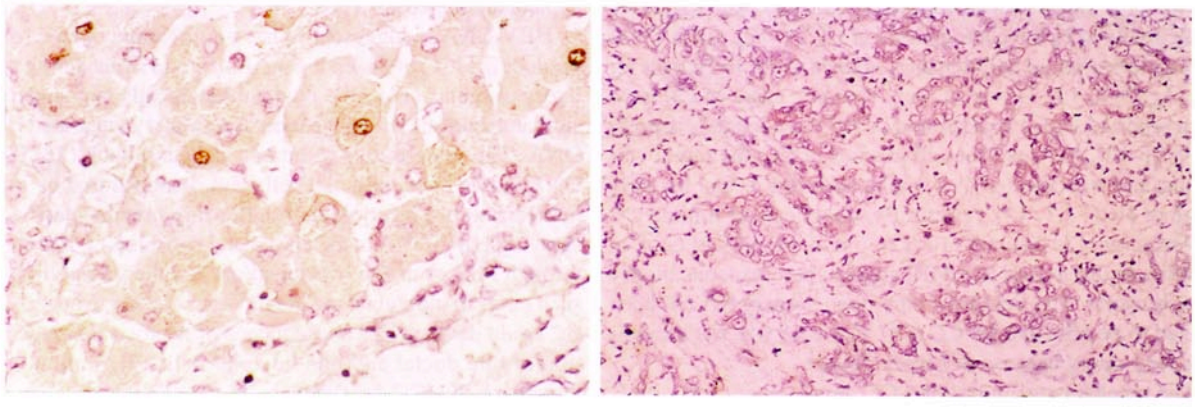
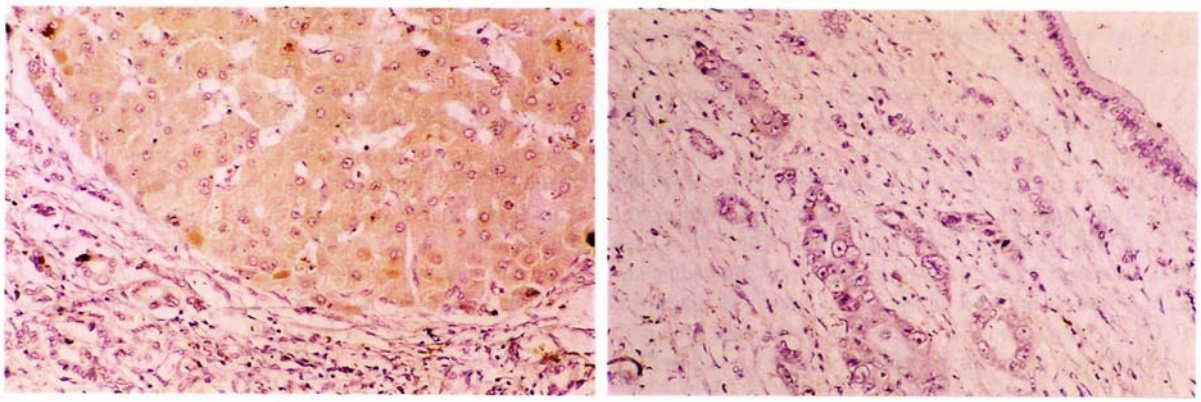


Figure 3. A) HBcAg in nuclei of some non-cancerous liver parenchymal cells (Avidin-biotin-peroxidase complex method, slightly counterstained with hematoxylin, ×400). B) HBcAg is not expressed in the cancer tissue (Avidin-biotin-peroxidase complex method, slightly counterstained with hematoxylin, ×200).



A B

Figure 4. A) Non-cancerous liver parenchymal cells are negative for HBX (Antibodies to 70646, HBX B, D and E, Ref.17, ×200). B) The cance tissues are negative for HBX (Antibodies to 70646, HBX B, D and E, Ref.17, ×200).

complex (ABC) method (16). The noncancerous liver parenchyma showed posthepatitic macronodular cirrhosis with pronounced proliferation of bile ductules. Immunohistochemistry for the viral surface (HBsAg), core (HBcAg) and HBX was performed using mouse monoclonal anti-HBsAg 3E7 (Dako, Copenhagen, Denmark), rabbit anti-HBcAg (B0586, Dako) and rabbit anti-HBX 70646 as well as mouse monoclonal antibodies HBXAb-B and D (17), respectively. Expression of HBsAg and HBcAg (Fig. 3A) was evident in noncancerous liver parenchyma, but was absent in the tumor (Fig. 3B). HBX-immunoreactivity was neither observed in the cancerous tissue (Fig. 4B) nor in the liver parenchyma (Fig. 4A).

Discussion

Recently, infection with hepatitis C virus has been suggested to be involved in the pathogenesis of CC (9, 10), since CC is found more often in individuals seropositive for HCV than for HBsAg (5, 6, 10). However, only sporadic cases of CC have been reported with a background of HBV-associated cirrhosis (7, 9, 11–13). In two of these five cases of CC, the tumor was minute and was detected by a similar approach as that for early stage of HCC (11, 12). A definitive diagnosis of minute CC before surgery is difficult (10–12), but in the present case, in addition to ultrasound and MRI studies, percutaneous biopsy proved useful. The treatment other than surgery has not been proved to be effective for CC (18). Our case was not a candidate for surgery because of hepatic failure.

The mechanisms underlying CC development are largely unknown. No studies have been reported about the mechanism which explain the relation between hepatitis B virus infection and carcinogenesis of CC. In an immunohistochemical study using rabbit anti-HBX antisera, Wang et al observed frequent, strong immunoreactivity in non-neoplastic and neoplastic bile duct epithelial cells, in addition to parenchymal cells, in some HBV-infected liver specimens, and suggested HBV to be a contributory factor also for the malignant transformation of bile duct cells (15). However, other groups reported that even in the liver parenchyma and HCC, only a small number of cells express HBX in chronic HBV infection (19–21). In a recent comparative study, Su et al found pronounced differences among 11 anti-HBX antibodies collected from 5 laboratories and demonstrated non-specificity for some of these tested (17), which may partly explain these discrepancies. In the present study, we did not find HBX-immunoreactivity in neoplastic, non-neoplastic hepatocytes, or cholangiocytes using a panel of antibodies whose specificity and conjugation capacity have been documented (17), while HBsAg and HBcAg were observed in the surrounding parenchyma. Expression of HBsAg and HBcAg in liver parenchymal cells decreases remarkably during hepatocarcinogenesis (17).

HBV-DNA is suggested to have a direct oncogenic role in producing HCC by its endogenous *cis*- and *trans*-acting regulatory elements (14). Bile duct epithelial cells may undergo a similar neoplastic transformation, if they carry integrated HBV-DNA. The integrations of human (22) and duck (23) HBV-DNA in bile duct epithelial cells was demonstrated by in situ hybridization. The etiologic relationship may be clarified by showing HBV-DNA in CC tissue and surrounding liver tissue through PCR or in situ hybridization. Another possibility with such cases in individuals with HBV-associated cirrhosis is that CC could develop as a consequence of necrosis and regeneration of bile ductal and bile ductular cells (7), similar to the hypothesis of HCC development. In the present case, non-cancerous tissue showed posthepatitic cirrhosis with pronounced proliferation of bile ductule, and CC could have arisen from such proliferating cells. Interestingly, bile duct proliferation is associated with periductal and periductular fibrosis resembling mucous cholangiofibrosis in rodents, which has been shown to be a frequent precursor of cholangiocellular neoplasms (24).

Acknowledgements: The authors are grateful to Dr. Malcolm Moore for his critical reading of the manuscript.

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□ CASE REPORT □

Insulinoma with Subsequent Association of Zollinger-Ellison Syndrome

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Abstract

We report a patient with insulinoma associated with Zollinger-Ellison syndrome. A 67-year-old woman was first admitted to our hospital for an abdominal mass. Abdominal computed tomography (CT) revealed a large pancreatic tumor, which was then diagnosed as an unresectable pancreatic adenocarcinoma. At the age of 71, she presented symptoms of hypoglycemia. Fasting blood glucose was 21 mg/dl and plasma immunoreactive insulin level was 846 μU/ml. Plasma gastrin, glucagon, vasoactive intestinal polypeptide and somatostatin levels were all normal. At the age of 73, hypoglycemic attacks occurred more frequently and she was admitted to our hospital. Abdominal CT scan showed multiple liver metastases. Chemotherapy with 5-fluorouracil and doxorubicin was performed. Three months later, she had an emergency laparotomy because of a perforated duodenal ulcer. Plasma gastrin level was 1,960 pg/ml at that time. Gastric hypersecretion was well controlled with a proton pump inhibitor (lansoprazole) but she died of widespread cancer dissemination 8 years after her first admission. On autopsy, histologic examination revealed a mixed acinar-endocrine carcinoma of the pancreas. Immunohistochemical stains were positive for insulin, gastrin, and α_1-antitrypsin.

(Internal Medicine 40: 386–390, 2001)

Key words: insulin, gastrin, multiple hormone syndrome, mixed acinar-endocrine carcinoma

Introduction

Patients with islet cell tumors may present multiple clinical features if tumor cells secrete more than one hormone (1, 2). However, there are a few reports of patients with endocrine pancreatic tumors that secrete a second or even a third hormone with subsequent development of new clinical symptoms (1, 3). In the present paper, we report a patient having insulinoma with hypoglycemia who later developed Zollinger-Ellison syndrome. Autopsy revealed that the pancreatic tumor was a mixed acinar-endocrine carcinoma of the pancreas.

Case Report

A 67-year-old woman was admitted to our hospital because of an abdominal mass detected by ultrasonography at a periodical medical checkup. The physical examination revealed an abdominal mass. She had no complaints of endocrine tumor-related symptoms. Computed tomography (CT) showed a mass in the body of the pancreas with invasion of the celiac and superior mesenteric arteries (Fig. 1). Endoscopic retrograde cholangiopancreatography showed an abrupt obstruction of the main pancreatic duct in the body of the pancreas (Fig. 2). We diagnosed her abdominal mass as an unresectable pancreatic cancer. At the age of 71, she presented with hypoglycemia. The physical examination was normal except for a palpable mass in the upper abdomen. Fasting blood glucose was 21 mg/dl and plasma immunoreactive insulin was 846 μU/ml (Table 1). Plasma levels of gastrin, glucagon, vasoactive intestinal polypeptide (VIP) and somatostatin were normal (Table 2). Contrast-enhanced CT showed a hypervascular tumor in the body and tail of the pancreas (Fig. 3A). We diagnosed her tumor as insulinoma. At the age of 73, she was admitted to our hospital again owing to frequent hypoglycemic attacks. Abdominal CT showed multiple liver metastases (Fig. 3B). Chemotherapy with 5-fluorouracil (5-FU) and doxorubicin was given. Three months later, she suddenly complained of severe abdominal pain and underwent emergency laparotomy with diagnosis of perforated duodenal ulcer. Plasma gastrin (1,960 pg/ml) and glucagon (1,590 pg/ml) levels were significantly elevated. Growth hormone (GH), adrenocorticotropic hormone (ACTH) and prolactin were slightly elevated. Other pituitary and parathyroid hormone levels were normal (Table 2). Lansoprazole (a proton pump inhibitor, 30 mg/day) inhibited gastric hypersecretion and improved her symptoms. Treatments with octreotide, diazoxide, and 5-FU and doxorubicin proved

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Received for publication June 16, 2000; Accepted for publication October 30, 2000
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