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Case Study

Changes in Laboratory Test Results and Diagnostic Imaging Presentation before the Detection of Occupational Cholangiocarcinoma

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Abstract: Changes in Laboratory Test Results and Diagnostic Imaging Presentation before the Detection of Occupational Cholangiocarcinoma: Shoji KUBO, *et al*. Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine—Objectives: A cholangiocarcinoma outbreak among workers of an offset color proof-printing department in a printing company was recently reported. It is important to understand the clinical course leading to occupational cholangiocarcinoma development for investigation of the carcinogenesis process and for surveillance and early detection. We evaluated the changes in laboratory test results and diagnostic imaging presentation before the detection of cholangiocarcinoma. **Methods:** We investigated the changes in laboratory test results and diagnostic imaging presentation before the detection of cholangiocarcinoma in 2 patients because the data were available. **Results:** The clinical courses observed in the 2 participating patients showed persistent elevation of serum γ-glutamyl transpeptidase levels with or without elevated serum levels of alanine aminotransferase and/or aspartate aminotransferase before cholangiocarcinoma detection. Dilatation of the bile ducts without tumor-induced stenosis was observed several years before cholangiocarcinoma detection and progressed gradually in both patients. The serum concentration of carbohydrate 19-9 also increased prior to cholangiocarcinoma detection in both patients. Eventually, observation of stenosis of the bile duct and a space-occupying lesion strongly suggested cholangiocarcinoma. Pathological examination of the resected specimens showed chronic bile duct injury and neoplastic lesions, such as "biliary intraepithelial neoplasia" and "intraductal papillary neoplasm of the bile duct" in various sites of the bile ducts, particularly in the dilated bile ducts. **Conclusions:** The changes in laboratory test results and diagnostic imaging might be related to the development of cholangiocarcinoma. It is important to monitor diagnostic imaging presentation and laboratory test results in workers with extended exposure to organic solvents.
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Key words: Carbohydrate 19-9, Dilated bile ducts, γ-Glutamyl transpeptidase, Occupational cholangiocarcinoma, Organic solvent

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Recently, a cholangiocarcinoma outbreak among former and current workers of an offset color proof-printing department of a printing company was reported in Japan[1, 2]. The disease was diagnosed in relatively young workers from 25 to 45 years old with a mean age of 36 years, and the observed incidence was unusually high in the abovementioned department (17 of 111 workers diagnosed)[2]. In that department, various chemicals including organic solvents, such as 1,1,1-trichloroethane, dichloromethane, and 1,2-dichloromethane (DCP), were used to clean ink residues. An experimental reconstruction of the working environment conducted by the Japanese National Institute of Occupational Safety and Health suggested that these workers were exposed to high concentrations of organic solvents[3]. Dichloromethane is classified as group 2B (possibly carcinogenic to humans) according to the International Agency for Research on Cancer[4]. The Japanese Ministry of Health, Labour and Welfare reported that biliary tract cancer was most probably

caused by long-term exposure to high DCP concentrations and that this type of cholangiocarcinoma was recently classified as an occupational disease[5].

It is important to understand the clinical course leading to the development of such an occupational cholangiocarcinoma for investigation of the carcinogenesis process and to optimize clinical surveillance for early detection. Of the 17 patients with occupational cholangiocarcinoma in the previously mentioned printing company, complete information describing the changes in laboratory test results and diagnostic imaging presentation before cholangiocarcinoma detection was available for 2 patients. In the present report, we described these changes to understand the clinical characteristics of these 2 patients. This study was approved by the ethics committee of Osaka City University, and both patients provided written informed consent.

Case Presentation

Case 1

Cholangiocarcinoma was diagnosed when the patient was 39 years old. He was not a habitual alcohol consumer and did not receive prior treatment. The diagnosis was made 13 years and 3 months after he started working at the printing company, where he was exposed to DCP for 7 years and 4 months. He received treatment for acute hepatitis 1 month after starting at the company (data not available). At 9 years and 4 months before cholangiocarcinoma detection, his laboratory test results were within the reference range (Fig. 1A). However, at 8 years and 4 months prior to cholangiocarcinoma detection, an elevated level of serum γ-glutamyl transpeptidase (γ-GTP, 101 U/*l*; reference value ≤86 U/*l*) was first noted, and it continued to increase gradually. At 3 years and 9 months before diagnosis, his serum levels of aspartate aminotransferase (AST, 50 U/*l*; reference value ≤38 U/*l*) and alanine aminotransferase (ALT, 62 U/*l*; reference value ≤43 U/*l*) were elevated. Magnetic resonance cholangiopancreatography (MRCP) taken at 2 years before diagnosis showed multiple localized dilatations of the peripheral bile ducts without tumor-induced stenosis of the bile ducts in the posterior segment (Fig. 1B). The patient was suspected to have primary sclerosing cholangitis (PSC).

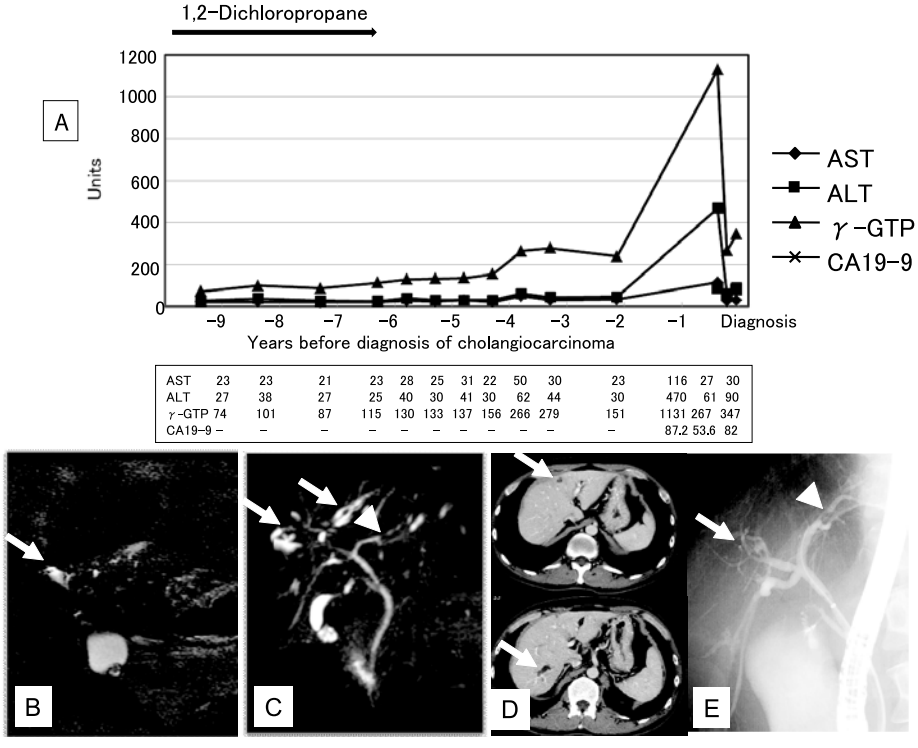

Fig. 1. Changes in laboratory test results and diagnostic imaging presentation before the diagnosis of cholangiocarcinoma in case 1. (A) Laboratory test results. (B) Magnetic resonance cholangiopancreatography (MRCP) 2 years before cholangiocarcinoma diagnosis. (C) MRCP 4 months before detection[2]. (D) Computed tomography (CT) at the time of cholangiocarcinoma diagnosis. (E) Endoscopic retrograde cholangiopancreatography (ERCP) at the time of cholangiocarcinoma diagnosis. Arrows show localized dilatation of the bile ducts. Arrowheads show stenosis of the bile duct (B2).

At 4 months before the cholangiocarcinoma diagnosis, he visited a hospital for abdominal pain, jaundice and acholic stool. His serum levels of γ-GTP, ALT and aspartate aminotransferase (AST) were elevated due to acute cholangitis. MRCP showed that the previously noted localized dilatation of the bile ducts was progressing, and a stenosis of the bile duct (B2) was suspected (Fig. 1C). His serum level of carbohydrate 19-9 (CA 19-9, reference value, ≤37 U/m*l*) was first measured and elevated (87.2 U/m*l*) 4 months before the diagnosis of cholangiocarcinoma. Ultrasonography showed the dilated bile ducts in the posterior segment (B7) and high echoic change of the bile duct walls in the lateral segment. Dynamic computed tomography (CT) showed dilatation of the bile ducts in various parts of the liver (Fig. 1D). Endoscopic retrograde cholangiopancreatography (ERCP) showed stenosis of the bile duct (B2) and dilatation of the bile ducts in the posterior segment (Fig. 1E). Brushing cytology of bile obtained from the stenotic site suggested adenocarcinoma, and cholangiocarcinoma was eventually diagnosed. At admission, laboratory test results showed elevated serum levels of ALT (57 U/*l*; reference value, ≤33 U/*l*), γ-GTP (347 U/*l*; reference value, ≤60 U/*l*), and CA19-9 (105 U/m*l*; reference value, ≤37 U/m*l*). The liver functional reserve was normal. The results of hepatitis B surface antigen and hepatitis C virus antibody titer tests were negative. His body mass index at admission was 24.3.

During surgery, intraoperative ultrasonography showed dilatation of the bile ducts (B7) with papillary tumors inside the bile ducts. Therefore, left lobectomy and segmentectomy (segment 7) were performed. Pathological examination of the resected specimens was performed. Precancerous or early-staged cancer lesions such as biliary intraepithelial neoplasia (BilIN)-2/3 and intraductal papillary neoplasm of the bile ducts (IPNB) were evaluated[6, 7]. Pathological examination showed chronic bile duct injury including sclerosis of large and medium-sized bile ducts (Fig. 2A) and intraepithelial neoplastic changes corresponding to BilIN-2/3 lesions at the various sites of the dilated and non-dilated bile ducts (Fig. 2B) and in the peribiliary glands. Focally, papillary lesions corresponding to IPNB were observed in the dilated intrahepatic bile ducts (Fig. 2C), and some parts of the IPNB showed cancer cell infiltration into the portal tract and perineural invasion (invasive IPNB or intraductal growth type of intrahepatic cholangiocarcinoma, Fig. 2D). The pathological examination of segment 7 showed such papillary changes in the dilated bile ducts (Fig. 2E), and some of them showed considerable mucin secretion with infiltration into the surrounding tissue and focal rupture. Pathological examination of the background liver showed nonspecific reactive changes such as mild portal inflammatory cell infiltration and fibrosis.

Case 2

Cholangiocarcinoma was diagnosed when the patient was 31 years old. He was not a habitual alcohol consumer and did not receive prior treatment. The diagnosis was made 12 years and 6 months after he started working at the printing company, where he was exposed to DCP for 6 years and 6 months. He retired from this position because extremely elevated levels of serum γ-GTP (1,182 U/*l*), AST (84 U/*l*), and ALT (144 U/*l*) were noted (although accurate reference values were unclear, the results were abnormally high) 6 years before the diagnosis of cholangiocarcinoma. His serum levels of γ-GTP, AST and ALT gradually decreased after his retirement (Fig. 3A). He started to receive ursodeoxycholic acid (600 mg/day) for liver dysfunction 3 years and 6 months before the diagnosis of cholangiocarcinoma. A CT scan performed at 5 years before cholangiocarcinoma diagnosis showed localized dilatation of the bile ducts in the posterior segment without tumor-induced stenosis of the bile duct (Fig. 3B). MRCP at 3 years and 6 months and at 8 months before cholangiocarcinoma diagnosis indicated that the number of localized bile duct dilatations and the degree of dilatation were increasing (Fig. 3C, 3D). Further, a protruded lesion was discovered in the hepatic duct (Fig. 3D, 3E). The patient's serum level of CA19-9 was first measured at 4 years and 10 months before cholangiocarcinoma diagnosis. Although his serum level of CA 19-9 increased at 4 years and 7 months (40 U/*l*, reference value, ≤37 U/m*l*) and at 3 years and 7 months (70 U/m*l*) before diagnosis, his serum level then decreased to the reference range. The serum level of CA19-9 started increasing again at 1 year and 5 months (43 U/*l*) before diagnosis (Fig. 3A). He started to receive ursodeoxycholic acid (600 mg/day) for liver dysfunction 3 years and 6 months before the diagnosis of cholangiocarcinoma. At admission, laboratory test results showed elevated serum levels of γ-GTP (75 U/*l*; reference value, ≤60 U/*l*), and CA19-9 (501 U/m*l*; reference value, ≤37 U/m*l*). A space-occupying lesion then appeared in the posterior segment of the liver (Fig. 3F). ERCP showed obstruction of the bile ducts in the posterior segment and a protruding lesion in the hepatic duct (Fig. 3G). The patient was diagnosed with a mass-forming type of intrahepatic cholangiocarcinoma and papillary type of extrahepatic cholangiocarcinoma at the hepatic duct. The results of hepatitis B surface antigen and hepatitis C virus antibody titer tests were negative. The patient's body mass index at admission was 16.2. He underwent right lobectomy and resection of the common hepatic and bile

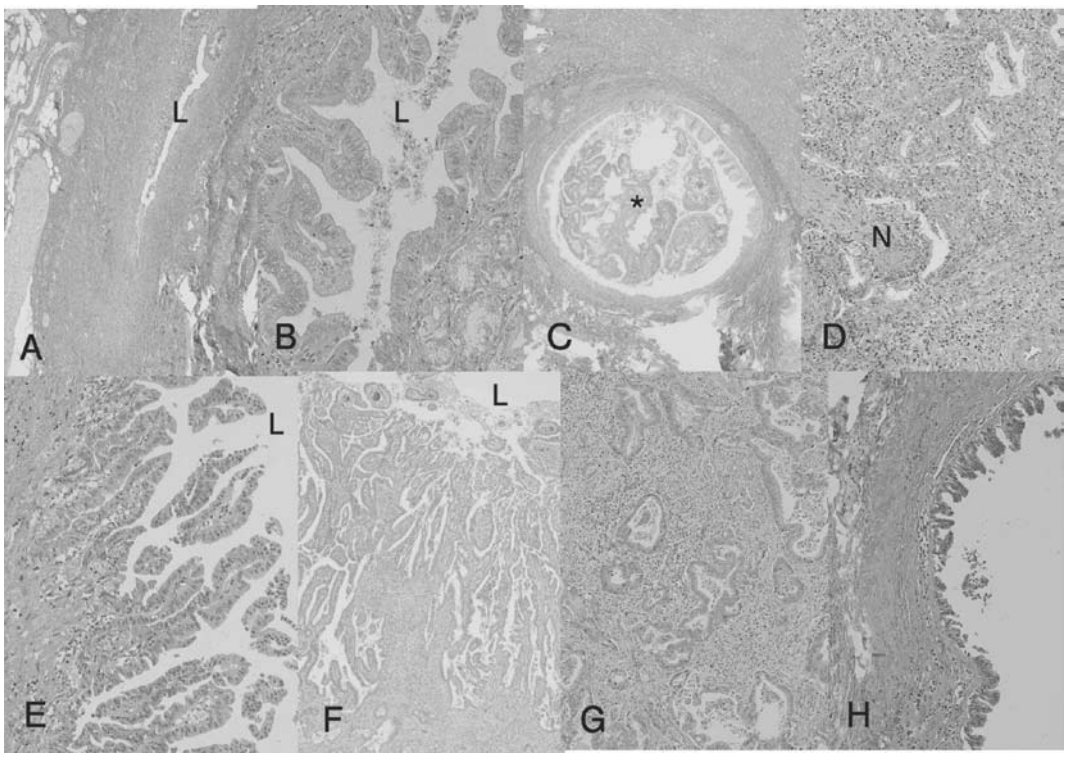

Fig. 2. Pathological findings of the resected specimens.
A, The large bile duct shows fibrous thichening of the duct wall and periductal tissue and erosion of the linig epithelia. L, bile duct lumen. HE. B, BilIN-2 lesion. L, Bile duct lumen. HE. C, The dilated bile duct contains neoplastic lining epithelia and a papillary neoplastic lesion corresponding to IPNB (*). HE. D, Cancer cell infiltrations and perineural invasion are evident in the portal tract. N, Nerve fiber. HE. E, Large bile ducts in the S7 shows papillary projection with atypical features, corresponding to IPNB with severe atypia in the lumen (L). HE. F, The large bile ducts contains a papillary neoplasm in the dilated bile ducts and this neoplasm shows infiltration into the surrounding tissue. L, Bile duct lumen. HE. G, The infiltrated part shows papillotubular adenocarcinoma. HE. H, The lining epithelium shows micropapillary features and stratification of nuclei, corresponding to BilIN-3. HE.

ducts with anastomosis of the left hepatic duct and the jejunum (Roux-en-Y procedure). Pathological examination of the resected specimens showed luminal dilatation, and papillary carcinoma was observed in the ductal lumen (Fig. 2F). This well-differentiated carcinoma infiltrated into the periductal tissue (Fig. 2G), forming a mass (mass-forming type of intrahepatic cholangiocarcinoma). In the dilated bile ducts, BilIN 2/3 lesions were observed (Fig. 2H). The medium and large-sized bile ducts showed chronic bile duct injury including nonspecific degenerative epithelial lesions and fibrosis, and the background liver showed nonspecific reactive changes similar to case 1.

The clinical courses of both patients showed persistent elevation of serum levels of γ-GTP with or without elevated serum levels of AST and/or ALT. Dilatation of the bile ducts without tumor-induced stenosis was detected several years before the diagnosis of cholangiocarcinoma in both patients. The serum level of CA19-9 also increased before cholangiocarcinoma diagnosis in both patients. Eventually, the stenosis of the bile duct, space-occupying lesion and protruding lesion in the bile duct strongly suggested cholangiocarcinoma. Pathological examination showed chronic bile duct injury and neoplastic lesions, such as BilIN and IPNB, in the various sites of the bile ducts, particularly in the dilated bile ducts.

Discussion

Among former and current workers of the offset color proof-printing department of a Japanese printing company, cholangiocarcinoma developed at an extremely high incidence[1, 2]. These workers were exposed to high concentrations of chlorinated organic solvents for a prolonged period. Thus, exposure to chlorinated organic solvents, including DCP, is thought to be a highly probable cause of cholangiocarcinoma development. In the 2 patients described here, liver

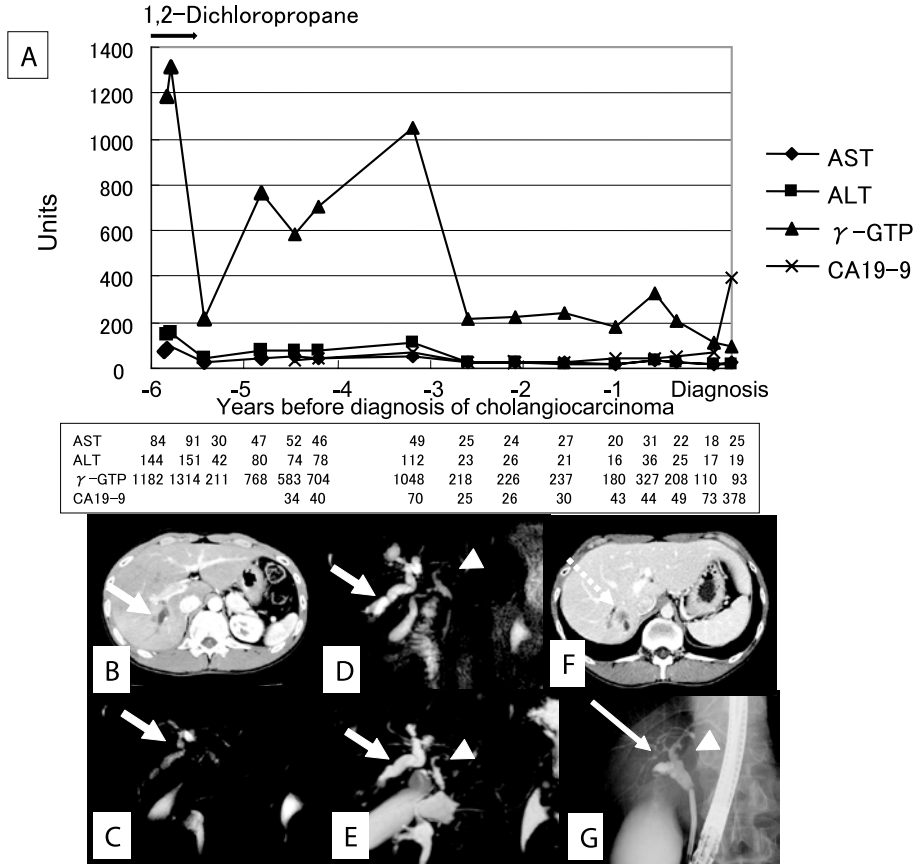

Fig. 3. Changes in laboratory test results and diagnostic imaging presentation before the diagnosis of cholangiocarcinoma in case 2. (A) Laboratory test results. (B) CT at 5 years before diagnosis. (C) MRCP at 3 years and 6 months before diagnosis. (D) MRCP at 8 months before diagnosis. (E) MRCP at 3 months before diagnosis. (F) CT at the time of cholangiocarcinoma diagnosis[2]. (G) ERCP at the time of diagnosis. Short arrows show localized dilatation of the bile ducts. Long arrow shows obstruction of the bile ducts in the posterior segment. The dotted arrow shows a mass-forming cholangiocarcinoma. Arrowheads show the protruded lesion in the hepatic duct.

dysfunction, including an elevated serum level of γ-GTP, was detected during a regular health examination performed several years before the diagnosis of cholangiocarcinoma. The serum levels of γ-GTP, AST, and ALT increased gradually during employment at the company in patient 1. On the other hand, the levels gradually decreased after the second patient's retirement from the company. International chemical safety cards[8] indicate that DCP may affect the liver. These findings suggest that the observed liver dysfunction might be related to DCP exposure.

Pathological examination of the 2 patients showed chronic bile duct injury, including bile duct sclerosis, and neoplastic lesions, such as BilIN 2/3 and IPNB, in various sites of the bile ducts in the noncancerous hepatic tissues of both patients. In a study including all 17 patients with occupational cholangiocarcinoma, the serum levels of γ-GTP were elevated in all patients, and chronic bile duct injury was observed in all 8 patients for which pathological examination could be performed[2]. These findings indicate that an elevated serum level of γ-GTP might be related to chronic bile duct injury resulting from exposure to DCP. Therefore, at regular health examinations for workers exposed to organic solvents, it is important to monitor the serum levels of γ-GTP, AST and ALT, which may indicate chronic bile duct injury.

Conversely, localized dilatation of the bile ducts without tumor-induced stenosis was an important characteristic observed in the diagnostic imaging of the 2 patients. Similar findings were observed in other patients with occupational cholangiocarcinoma[2]. Pathological examination showed that the dilated bile ducts were related to chronic bile duct injury and neoplastic lesions, such as BilIN and IPNB. These imaging findings, especially of MRCP, in the

2 patients were similar to those observed in PSC[9], including multifocal, intrahepatic bile duct strictures alternating with normal-caliber ducts, which sometimes produce a beaded appearance. It is important to distinguish changes in the bile ducts induced by an organic solvent from PSC. In the 2 patients in this study, diagnostic imaging, including CT and magnetic resonance imaging, eventually showed bile duct stenosis, space-occupying lesions and a protruding lesion in the bile duct. A previous study indicated that occupational cholangiocarcinoma might result from chronic bile duct injuries progressing into precancerous or early cancerous lesions (BilIN and/or IPNB) at various sites of the bile ducts and eventually developing into invasive cholangiocarcinoma[2], which is similar to cholangiocarcinoma in patients with hepatolithiasis, PSC or liver flukes[10–12]. Thus, it is important to monitor changes in the shape of the bile ducts. With regard to imaging analyses, the progression of localized dilatations of the bile ducts should be closely monitored because they probably have malignant potential or malignancy. Further, both mass lesions with or without dilatation of the peripheral bile duct and dilatation and/or stenosis of the bile ducts are important findings for detecting cholangiocarcinoma.

Early cholangiocarcinoma detection is essential because surgery is the only potential curative treatment[13, 14]. Therefore, it is necessary to monitor diagnostic imaging and laboratory test results, including the levels of γ-GTP, AST and ALT and the serum level of CA19-9, for workers with extended exposure to high concentrations of organic solvents.

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