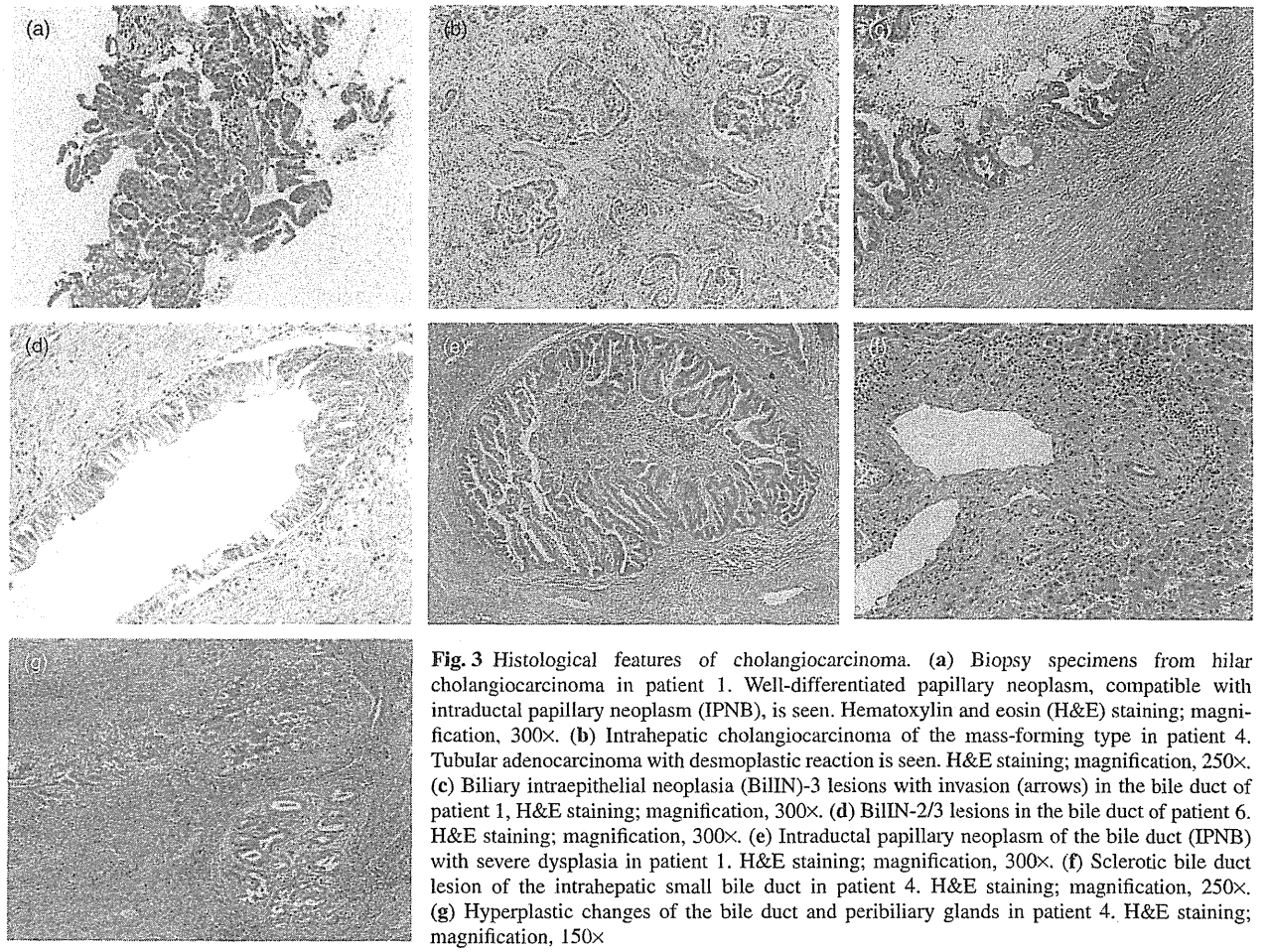

Fig. 3 Histological features of cholangiocarcinoma. (**a**) Biopsy specimens from hilar cholangiocarcinoma in patient 1. Well-differentiated papillary neoplasm, compatible with intraductal papillary neoplasm (IPNB), is seen. Hematoxylin and eosin (H&E) staining; magnification, 300×. (**b**) Intrahepatic cholangiocarcinoma of the mass-forming type in patient 4. Tubular adenocarcinoma with desmoplastic reaction is seen. H&E staining; magnification, 250×. (**c**) Biliary intraepithelial neoplasia (BilIN)-3 lesions with invasion (arrows) in the bile duct of patient 1, H&E staining; magnification, 300×. (**d**) BilIN-2/3 lesions in the bile duct of patient 6. H&E staining; magnification, 300×. (**e**) Intraductal papillary neoplasm of the bile duct (IPNB) with severe dysplasia in patient 1. H&E staining; magnification, 300×. (**f**) Sclerotic bile duct lesion of the intrahepatic small bile duct in patient 4. H&E staining; magnification, 250×. (**g**) Hyperplastic changes of the bile duct and peribiliary glands in patient 4. H&E staining; magnification, 150×

adenocarcinoma was confirmed by needle biopsy of the liver. Of the remaining four patients (patients 2, 4, 6, and 8), adenocarcinoma was confirmed by surgical specimens. An autopsy was performed on one patient (patient 1).

With regard to the histological subtypes, well- to poorly differentiated adenocarcinoma (Fig. 3b) was identified in the four patients diagnosed with the mass-forming type of intrahepatic cholangiocarcinoma, and tubular adenocarcinoma was identified in two patients with hilar cholangiocarcinoma (patients 2 and 8).

BilIN-2/3 lesions with or without invasion were detected at various sites of the large intrahepatic bile ducts and/or hilar bile ducts and the peribiliary glands in three available surgical specimens (patients 2, 4, 6) and in one autopsy specimen (patient 1, Fig. 3c,d). IPNB lesions with severe dysplasia were detected in one patient (patient 1; Fig. 3e). In these same four patients (patients 1, 2, 4, 6), sclerosis of the bile duct with variable inflammatory cell proliferation, biliary epithelial injuries/focal bile duct loss, and biliary epithelial hyperplasia were also observed at various sites of the bile ducts in the noncancerous hepatic tissues (Fig. 3f,g). With regard to the pathology of the non-neoplastic liver tissue, we observed non-specific reactive changes or cholestatic changes secondary to obstruction or stenosis of the bile ducts affected by cholangiocarcinoma or radiation.

No cirrhotic changes or other hepatobiliary diseases were detected in the noncancerous hepatic tissues of the four patients (patients 1, 2, 4, 6).

Risk factors for cholangiocarcinoma

The laboratory test results, diagnostic imaging results, and/or pathological findings indicated that the nine patients did not have any known risk factors for developing cholangiocarcinoma, such as primary sclerosing cholangitis, hepatolithiasis, pancreaticobiliary maljunction, or infection with liver flukes (e.g., *Clonorchis sinensis* or *Opisthorchis viverrini*) [4, 9–13].

Table 4 Treatments and prognoses after diagnosis

Patient no.	Treatments	Prognosis
1	Chemotherapy, stenting, hyperthermia, radiotherapy, proton beam radiotherapy	3 years 3 months, dead
2	Extended right lobectomy, resection of extrahepatic duct, resection and reconstruction of portal vein, chemotherapy, radiation	3 years 5 months, alive
3	Proton beam radiotherapy, resection of metastatic lymph node (recurrence), chemotherapy	6 years 10 months, alive
4	Extended left lobectomy, resection of extrahepatic ducts	1 year 4 months, dead
5	Chemotherapy, stenting	8 months, dead
6	Partial resection of the liver	2 years, dead
7	Chemotherapy, stenting	5 years 7 months, alive
8	Extended left lobectomy, resection of extrahepatic ducts, resection and reconstruction of portal vein, chemotherapy	4 years 7 months, alive
9	Chemotherapy, stenting	1 year 6 months, dead

Treatment and prognosis

Surgical resection was performed in four patients (Table 4). Dissection or sampling of lymph nodes was performed in three patients (patients 2, 4, 8); one (patient 2) of the three patients exhibited metastasis to the lymph nodes around the common bile duct or the common hepatic artery. Portal invasion was diagnosed in two patients (patients 2, 8) by pathological examination. Neoadjuvant chemoradiotherapy with gemcitabine and adjuvant chemotherapy with gemcitabine and S-1 (tegafur/gimeracil/oteracil potassium) was administered to one patient (patient 2). In one patient (patient 6), chemotherapy with cisplatin and 5-fluorouracil was administered before surgery. Another patient (patient 3) received proton beam radiotherapy followed by chemotherapy (gemcitabine and cisplatin), according to his wishes. In the remaining four (patients 1, 5, 7, and 9) of the nine patients, chemotherapy (S-1, gemcitabine, and/or cisplatin) with stenting to treat the obstruction of the bile duct was performed because of the advanced stage of the disease, as indicated by multiple tumors in the liver and metastasis to the lymph nodes. One patient (patient 1) received hyperthermia, radiation, and proton beam radiation therapy in addition to chemotherapy.

Among the four patients who underwent surgical treatment, intrahepatic recurrence or local recurrence occurred in three patients (patients 4, 6, and 8). In these three patients, radiation (patient 4), second liver resection (patient 6), and chemotherapy (patient 8) was performed, respectively. In one patient who underwent proton beam radiotherapy and chemotherapy, surgical resection of the lymph node recurrence was performed (patient 3).

Two (patients 4, 6) of the four patients who underwent surgical treatment died of cholangiocarcinoma recurrence. Three (patients 1, 5, 9) of the remaining five patients died of cholangiocarcinoma. The survival time from the diagnosis of cholangiocarcinoma to death or the end of the study (February 2014) ranged from 255 to 2517 days (median, 825 days).

Comparison in clinicopathlogical findings between 17 patients at a printing company in Osaka and in nine patients at other printing companies

The mean age was significantly higher in the nine patients at other printing companies than in the 17 patients at a printing company in Osaka (Table 5). The proportions of patients with elevated serum activity of γ-GTP and elevated serum concentration of CA19-9 were not different between the groups. When the regional dilatation of intrahepatic bile ducts without tumor-induced obstruction were investigated in patients without hilar cholangiocarcioa or upper bile duct cancer (because the dilatation of intrahepatic bile duct due to tumor-induced obstruction tends to obscure this characteristic), the proportion of patients with the regional dilatation of intrahepatic bile ducts without tumor-induced obstruction were not different between the groups. The proportion of patients with intrahepatic cholangiocarcinoma and/or extrahepatic cholangiocarcinoma was not different between the groups. The preneoplastic or early preinvasive neoplastic lesions such as BilIN or IPNB, and chronic bile duct injury were detected in all patients examined in both groups.

Discussion

We previously reported on 17 patients with occupational cholangiocarcinoma who were former or current workers in an offset color proof-printing department at a printing company in Osaka, Japan [2]. These patients had been exposed to a high concentration of chlorinated organic

Table 5 Characteristic findings in 17 patients with occupational cholangiocarcinoma at a printing company in Osaka and in nine patients at other printing companies

Findings	Company in Osaka (n = 17)	Other companies (n = 9)	P
Age (years old, mean)	25–45 (36)	31–57 (44)	0.0046
Elevated γ-GTP	17	9	>0.999
Elevated CA 19-9	13	6	0.661
Regional dilated bile ducts[a]	5/10	2/4	>0.999
ICC:ECC:ICC+ECC	10:5:2	4:5:0	0.312
BilIN and/or IPNB[b]	8/8	4/4	>0.999
Chronic bile duct injury[b]	8/8	4/4	>0.999

γ-GTP γ-glutamyl transpeptidase, *BilIN* biliary intraepithelial neaoplasia, *CA 19-9* carbohydrate antigen 19-9, *ECC* extrahepatic cholangiocarcinoma, *ICC* intrahepatic cholangiocarcinoma, *IPNB* intraductal papillary neoplasm of the bile duct

[a] The lesions were examined in patients without hilar cholangiocarcinoma or upper bile duct carcinoma

[b] The pathological findings were revealed upon examination of the operative or autopsy specimens

solvents, including TCE, DCM, and DCP, over a long period of time (from 6 years and 1 month to 19 years and 9 months). DCM is classified under group 2B (possibly carcinogenic to humans) according to the International Agency for Research on Cancer [14]. As a result of the meticulous analysis of patients with this type of cholangiocarcinoma who have had a long history of the exposure to DCP and/or DCM, cholangiocarcinoma was recognized as a new occupational disease by the Japanese Ministry of Health, Labour and Welfare as of 1 October 2013 [3]. Until the end of 2013, the cholangiocarcinoma cases identified in 26 patients (17 original patients from one company and nine patients who worked at seven other printing companies) were classified as this new type of occupational cholangiocarcinoma.

The 17 original patients with occupational cholangiocarcinoma had the following characteristics: in addition to being relatively young, they had elevated serum activity of γ-GTP and serum concentration of CA19-9, regional dilatation of intrahepatic bile ducts without tumor-induced obstruction, precancerous or early-cancerous lesions at various parts of the bile ducts (BilIN and IPNB), and chronic bile duct injury (Table 5). Typically, cholangiocarcinoma occurs in the sixth or seventh decade of life, and the disease is rarely seen in younger patients. In the current patient series, patient ages ranged from 31 to 57 years (mean, 44 years). Although the mean age was significantly higher in the current series than in the previous series, the mean age in the current series also seemed to be young, similar to the reports in previous series (mean, 36 years). At cholangiocarcinoma diagnosis, the serum activity of γ-GTP was elevated in all 26 patients in both the previous and current studies (Table 5). In one patient in the previous series and two patients in the current series, we observed that the serum activity of γ-GTP increased gradually, with or without elevated AST and ALT activity, during regular health examination before the cholangiocarcinoma detection [15, 16]. These findings suggest that the observed liver dysfunction might be related to the patient's exposure to chlorinated organic solvents. The presence of regional dilatation of intrahepatic bile ducts without tumor-induced obstruction is a characteristic finding on diagnostic imaging [2]. The regional dilatation of intrahepatic bile ducts without tumor-induced obstruction was observed in five of the 10 patients in the previous series and in two of the four patients in the current series. In the current series, the primary and most invasive cholangiocarcinoma lesions were located in the large bile ducts, similar to the previous series. In the previous series, pathological examination revealed that BilIN-2/3 lesions and/or IPNB lesions with or without invasive portions were observed at various sites of the large intrahepatic bile ducts and/or hilar bile ducts and peribiliary glands. In addition, chronic bile duct injury, proliferative changes of the bile ducts, and bile duct sclerosis, those are characteristics of the pathological findings of the patients of the previous series [2, 17], were observed at various sites of the bile ducts in the noncancerous hepatic tissue. In the current series, operative or autopsy specimens were available for four patients. BilIN-2/3 and/or IPNB lesions with or without invasion, and chronic bile duct injury were observed at various sites of the bile ducts in all four patients. Thus, the pathological findings in the current series were similar to those of the previous series, although pathological examination could not be performed for all patients in either series. Taken together, most of the patients in the current study had characteristic findings of cholangiocarcinoma patients who had been exposed to chlorinated organic solvents.

Although five of the nine patients died of cholangiocarcinoma, four patients survived for more than 3 years after diagnosis. In all four of these patients, surgery, chemo-

therapy, radiotherapy, proton beam radiotherapy, and/or hyperthermia were performed. Thus, multidisciplinary treatment may promote long-term survival in these patients.

The patients in this study had a history of prolonged exposure to chlorinated organic solvent including DCM and DCP. Such chlorinated organic solvents might have played an important role in the development of cholangiocarcinoma. The current study indicates that chlorinated organic solvent-related cholangiocarcinoma developed in workers at several printing companies in Japan in addition to the prototype cases in Osaka [2]. Therefore, occupational cholangiocarcinoma is not limited to a single printing company in Osaka. The previous and current studies have demonstrated that a portion of cholangiocarcinoma cases appear to be caused by chemical exposure. As previously reported, it is necessary to monitor workers who are exposed to chlorinated organic solvents, and regular health examinations may contribute to early detection of occupational cholangiocarcinoma. Further analyses with larger sample sizes are necessary to clarify the mechanism by which occupational cholangiocarcinoma can arise.

Acknowledgments This study was supported in part by the Health and Labour Sciences Research Grants for Research on Occupational Safety and Health "The epidemiological and cause-investigated study of cholangiocarcinoma in workers of a printing company." We thank Drs K. Kimura and H. Uchiyama for their assistance with data collection.

Conflict of interest None declared.

Author contribution Study design: S.K., Y.N., and G.E. Acquisition of data: S.K., H.S., T.N., T.I., M. Abue, M. Aoki, K.N., M.U., S.H., T.F., Y.S., T.M., K.S, A.N., Y.O., K.T., T.K., T.H., A.S., H.N., M.T., and G.E. Clinical aspect of the study and analysis: S.K., M.K., S.Takemura, G.H., and S.Tanaka. Pathological examination and analysis: Y.K and Y.N. Data analysis: S.K., M.K., S. Takemura, Y.N., and G.E. Manuscript drafting: S.K., M.K., Y.N., and G.E. All authors reviewed the manuscript.

References

1. Kumagai S, Kurumatani N, Arimoto A, Ichihara G. Cholagiocarcinoma among offset colour proof-printing workers exposed to 1,2-dichloropropane and/or dichloromethane. Occup Environ Med. 2013;70:508–10.
2. Kubo S, Nakanuma Y, Takemura S, Sakata C, Urata Y, Nozawa A, et al. Case series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan. J Hepatobiliary Pancreat Sci. 2014;21:479–88.
3. Ministry of Health, Labour and Welfare. Occupational biliary tract cancer cases in Japan. 2013. http://www.mhlw.go.jp/english/policy/employ-labour/labour-standards/Occupational.html
4. World Health Organization Classifications of Tumours. International Agency for Research on Cancer (IARC). In: Bosman FT, Carneiro F, Hruban RH, Theise ND, editors. WHO classification of tumours of the digestive system, 4th edn. Lyon: World Health Organization Classifications of Tumours; 2010. p. 195–278.
5. Zen Y, Sasaki M, Fujii T, et al. Different expression patterns of mucin core proteins and cytokeratins during intrahepatic cholangiocarcinogenesis from biliary intraepithelial neoplasia and intraductal papillary neoplasm of the bile duct—an immunohistochemical study of 110 cases of hepatolithiasis. J Hepatol. 2006;44:350–8.
6. Zen Y, Adsay NV, Bardadin K, Colombari R, Ferrell L, Haga H, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. Mod Pathol. 2007;20:701–9.
7. Itatsu K, Zen Y, Ohira S, Ishikawa A, Sato Y, Harada K, et al. Immunohistochemical analysis of the progression of flat and papillary preneoplastic lesions in intrahepatic cholangiocarcinogenesis in hepatolithiasis. Liver Int. 2007;27:1174–84.
8. Nakanuma Y, Hoso M, Sanzen T, Sasaki M. Microstructure and development of the normal and pathologic biliary tract in humans, including blood supply. Micro Res Tech. 1997;38:552–70.
9. Parkin DM, Ohshima H, Srivatanakul P, Vatanasapt V. Cholangiocarcinoma: epidemiology, mechanisms of carcinogenesis and prevention. Cancer Epidemiol Biomarkers Prev. 1993;2:537–44.
10. Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. Semin Liver Dis. 2004;24:115–25.
11. Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. Lancet. 2005;366:1303–14.
12. Kubo S, Kinoshita H, Hirohashi K, Hamba H. Hepatolithiasis associated with cholangiocarcinoma. World J Surg. 1995;19:637–41.
13. Yamamoto S, Kubo S, Hai S, Uenishi T, Yamamoto T, Shuto T, et al. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. Cancer Sci. 2004;95:592–5.
14. IARC (International Agency for Research on Cancer). List of classification by cancer sites with sufficient or limited evidence in humans, volume 1 to 105. 2012. http://monographs.iarc.fr/ENG/Classification/index.php
15. Kubo S, Takemura S, Sakata C, Urata Y, Nishioka T, Nozawa A, et al. Changes in laboratory test results and diagnostic imaging presentation before the detection of occupational cholangiocarcinoma. J Occup Health. 2014; http://dx.doi.org/10.1539/joh.14-0002-CS.
16. Abue M, Suzuki M, Tsukamoto K, Aoki M, Kubo S. A case of intrahepatic cholangiocarcinoma with a printing company service history. Tando. (in press, in Japanese with English abstract).
17. Tomimaru Y, Kobayashi S, Wada H, Hama N, Kawamoto K, Eguchi H, et al. Intrahepatic cholangiocarcinoma in a worker at an offset color proof-printing company: an autopsy case report. Hepatol Res. 2014; doi: 10.1111/hepr.12363.

Osaka City Med. J. Vol. 60, 39-44, 2014

Cholangiocarcinoma in a Middle-aged Patient Working at a Printing Plant

Shogo Tanaka[1], Nobusuke Fukumoto[2], Kohichi Ohno[1], Sayaka Tanaka[3], Masahiko Ohsawa[3], Takatsugu Yamamoto[1], Yasuni Nakanuma[4], and Shoji Kubo[5]

Departments of Surgery[1] and Gastroenterology[2], Ishikiriseiki Hospital; Departments of Diagnostic Pathology[3] and Hepato-Biliary-Pancreatic Surgery[5], Osaka City University, Graduate School of Medicine; and Department of Human Pathology[4], Kanazawa University, Graduate School of Medicine

Abstract

A 39-year-old male with elevated serum transferases consulted our hospital in September 2010. Since 1999, he had worked at a printing company using organic solvents. Cholangiography revealed stenosis of the left hepatic duct with peripheral dilation, stricture of the right hepatic duct, and irregularity of the extrahepatic bile duct. As a preoperative diagnosis of sclerosing cholangitis and cholangiocarcinoma was made, extended left hepatectomy with resection of the extrahepatic bile duct and anastomosis of the anterior and posterior branches of the bile duct and the jejunum (Roux-en Y reconstruction) were performed. A histological examination showed papillary carcinoma of the medial hepatic bile duct with intraductal growth, and biliary intraepithelial neoplasia-2/3 lesions from the medial hepatic bile duct to the right hepatic and the common bile ducts. Chronic cholangitis was shown around the tumors. Postoperatively, the patient was treated with adjuvant chemo-radiation, and he is doing well 30 months after the operation, without recurrence. Unknown causes, including exposure to organic solvents, might have induced chronic bile duct injury and contributed to the development of cholangiocarcinoma.

Key Words: Biliary intraepithelial neoplasia; Intraductal papillary neoplasm of bile duct; Printing job; Thirties

Introduction

Cholangiocarcinoma (CC) is a malignant tumor that arises from the biliary epithelium in any part of the bile duct system, from the bile ductules to the ampulla of Vater[1]. There are several risk factors for CC, including primary sclerosing cholangitis, biliary-duct cysts (abnormal pancreatico-biliary junction), hepatolithiasis, parasitic infections (liver flukes), and hepatitis C and B virus infections[2-6]. However, even if these risk factors are present, CC rarely occurs before the age of 40[7,8]. In this report, we describe the case of a patient in his 30s who developed CC.

Received May 30, 2013; accepted October 15, 2013.
Correspondence to: Shogo Tanaka, MD.
Department of Surgery, Ishikiriseiki Hospital,
18-28 Yayoicho, Higashiosaka City 579-8026, Japan
Tel: +81-72-988-3121; Fax: +81-72-986-3860
E-mail: m8827074@msic.med.osaka-cu.ac.jp

Case Report

A 39-year-old Japanese male visited our hospital in September 2010 after detection of elevation of his serum transferases during a routine examination at his company. He had no symptoms or prior history and he did not drink alcohol or smoke. Since April 1999, he had worked at a printing factory and had been exposed to organic solvents, including 1,2-dichloropropane, which is classified as a category 2 agent (suspected human carcinogen) in the Globally Harmonized System of Classification and Labelling of Chemicals (GHS). These organic solvents had been used for the removal of ink for an average of 70 hours a week for at least 6 years. Laboratory data showed elevation of his serum aspartate aminotransferase (45 IU/L; normal <38 IU/L), alanine aminotransferase (92 IU/L; normal <44 IU/L) and γ-glutamyl transpeptidase (486 IU/L; normal <73 IU/L). The serum concentrations of total bilirubin and alkaline phosphatase were within the normal reference limits, and his serum was negative for hepatitis C antibodies, hepatitis B surface antigen, and antinuclear antigen. The level of serum carcinoembryonic antigen was slightly elevated (5.4 mg/mL; normal <4.8 ng/mL). His serum carbohydrate antigen 19-9 level was within normal limits (20.6 U/mL; normal <35.4 U/mL).

CT showed the dilation of the intrahepatic bile duct in the left hepatic lobe. Magnetic resonance imaging and magnetic resonance cholangiopancreatography showed stenosis of the left hepatic duct with dilation of the peripheral bile duct, right hepatic duct and common bile duct (Fig. 1). Endoscopic nasobiliary drainage was performed for bile sampling. Contrast injection from the endoscopic nasobiliary drainage tube showed stenosis of the left hepatic duct, stricture of the right hepatic duct, and irregularity of the common hepatic and common bile ducts (Fig. 2). The right intrahepatic bile ducts were narrow with the appearance of multifocal stricture. The cytology of bile revealed the presence of adenocarcinoma cells. Positron emission tomography revealed accumulation of F-18 fluorodeoxyglucose in the dilated bile duct in the medial segment (Fig. 3). Based on this evidence, we diagnosed (primary or secondary) sclerosing cholangitis (of unknown cause) and cholangiocarcinoma that originated around the left hepatic duct.

During laparotomy, the lymph nodes in the hepatoduodenal ligament, around the common hepatic artery, and in the dorsal pancreatic head were noted to be swollen; however, frozen sections of these lymph nodes were negative for cancer cells. After cutting of the retropancreatic bile duct (which

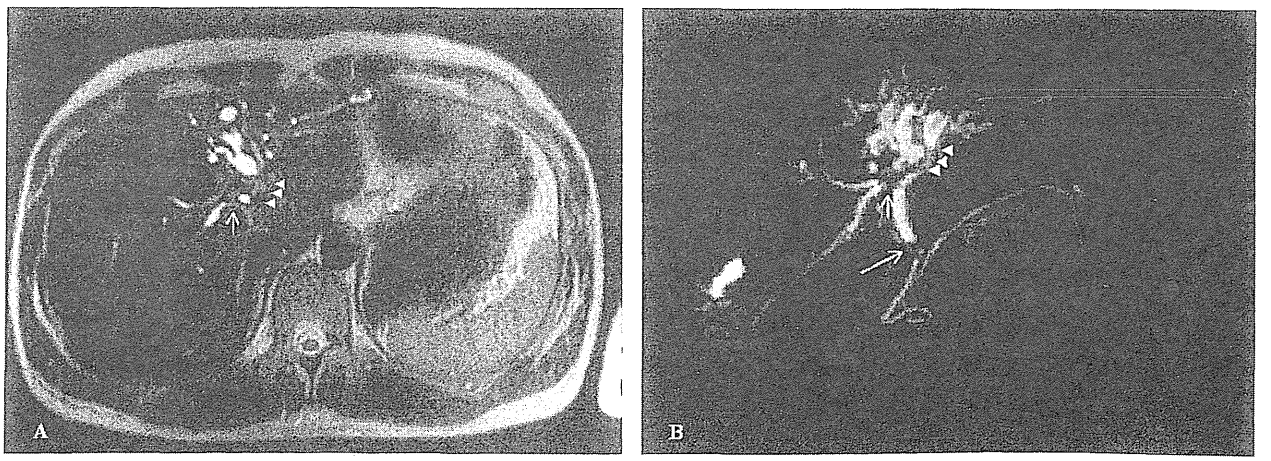

Figure 1. Magnetic resonance imaging (A) and cholangiopancreatography (B) showed stenosis of the left hepatic duct (arrowhead) with dilation of the intrahepatic bile duct in the medial and lateral segments, right hepatic duct (small arrow), and common bile duct (large arrow).

was also cancer cell negative), an intraoperative biliary scope from the stump showed no stenosis of the common bile duct, common hepatic duct, or right hepatic duct, and no suspected cancer lesions. However, frozen sections of the hilar bile duct and the bifurcation of the right hepatic duct revealed the presence of adenocarcinoma. The bifurcations of the right anterior and posterior branches were found to be negative for cancer cells. Therefore, we performed extended left hepatectomy with a resection of the extrahepatic bile duct, together with the anastomosis of the anterior and posterior branches of the bile duct and the jejunum (Roux-en-Y reconstruction). The cut surface of the resected specimen showed no suspected cancer lesions in the extrahepatic bile duct (Fig. 4). The left hepatic duct was stenosed and an intraductal protruded lesion was present in the bifurcation of the medial branch.

A histological examination revealed well-differentiated papillary carcinoma of the medial hepatic

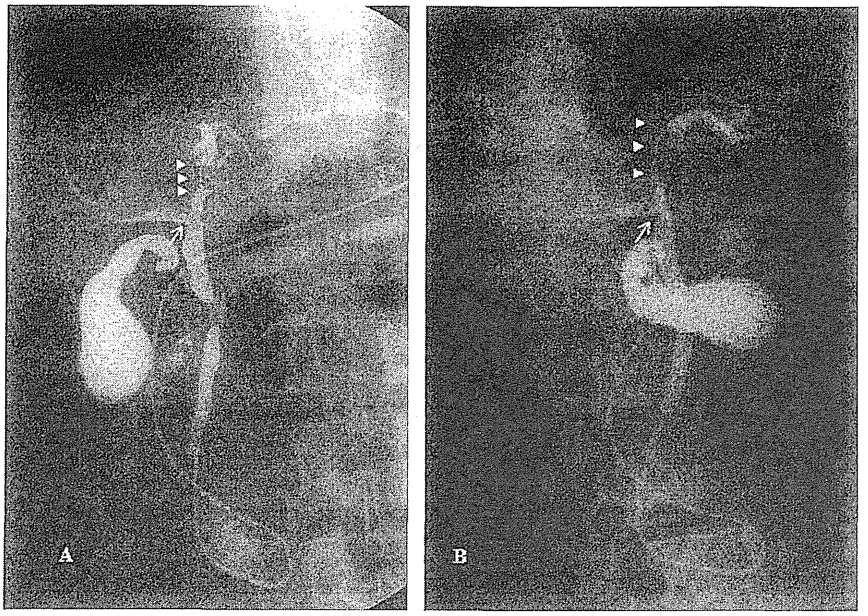

Figure 2. Contrast injection from an endoscopic nasobiliary drainage tube demonstrated obstruction of the left hepatic duct (arrowhead), stricture of the bifurcation of the right hepatic duct (small arrow), and irregularity of the common bile duct and common hepatic duct. The right intrahepatic bile ducts were narrow with apparent multifocal stricture. Anterior position (A) and right anterior oblique position (B).

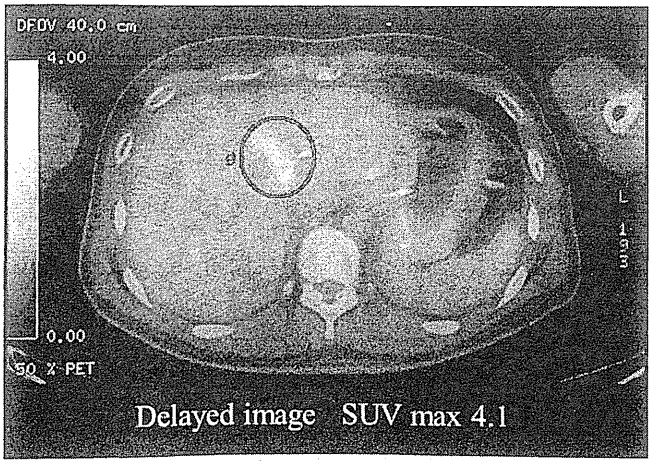

Figure 3. Positron emission tomography showed accumulation of F-18 fluorodeoxyglucose (FDG) in the left hepatic duct and dilated medial branch. Delayed image was obtained 2 hours after intravenous FDG injection.

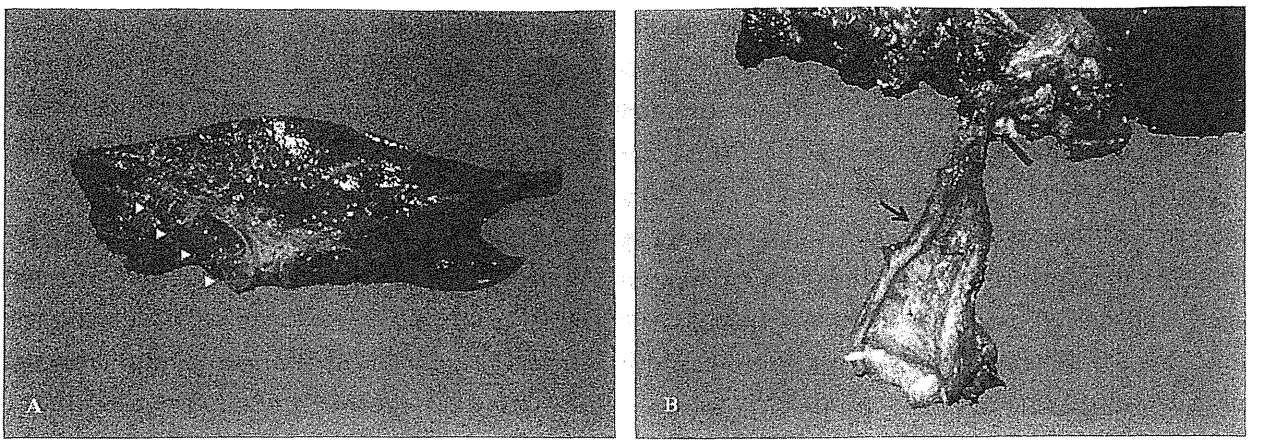

Figure 4. A cut surface of the resected specimen showed a slightly protruding lesion at the bifurcation of the medial bile duct branch (arrowhead) (A). The mucosa of the common bile duct and common hepatic duct was whitish and rough, but there was no clear cancerous lesion. The left hepatic duct was stenosed (large arrow). The small arrow indicates the first cut line of the stump of the right hepatic duct for preparation of a frozen section (B).

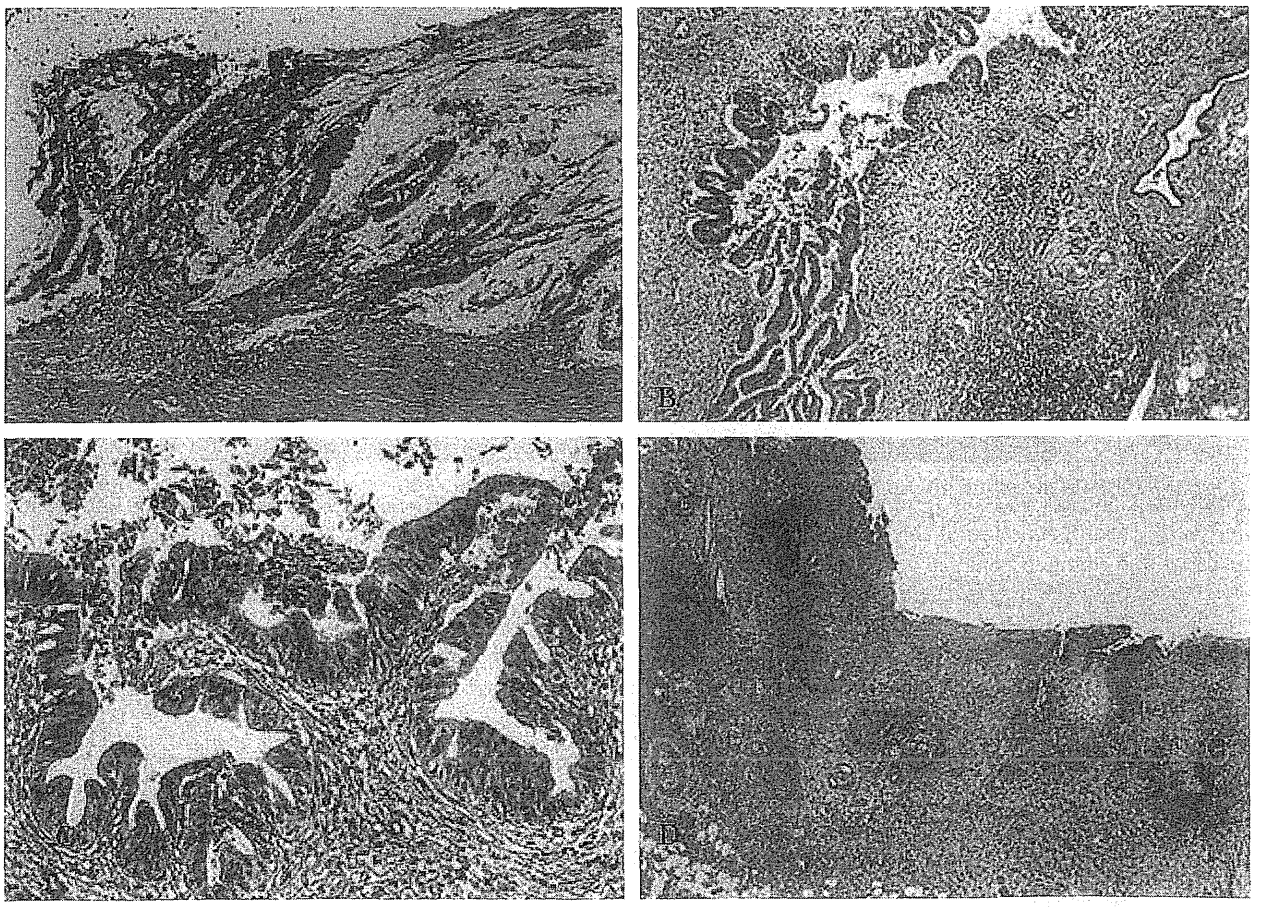

Figure 5. Histological examinations. A protruding tumor at the bifurcation of the medial hepatic bile duct was found to be well differentiated papillary carcinoma with the intraductal growth type (objective lens, ×10) (A). Biliary intraepithelial neoplasia-2/3 (high grade dysplasia and carcinoma in situ) lesions in the large intrahepatic bile duct with its glands. There was glandular proliferation with infiltration of lymphocytes around the lesions (objective lens, ×4) (B). Biliary intraepithelial neoplasia-2/3 (high grade dysplasia and carcinoma in situ) lesions in the large intrahepatic bile duct (objective lens, ×20) (C). Lymphocyte infiltration was diffusely present in the bile duct at the hepatic hilum (objective lens, ×4) (D).

bile duct with the intraductal growth type, which corresponds to an intraductal papillary neoplasm of bile duct (IPNB) with invasive carcinoma according to the 2010 WHO classification of intrahepatic cholangiocarcinoma[9] (Fig. 5A). Biliary intraepithelial neoplasia (BilIN)-2 (high grade dysplasia)/3 (carcinoma *in situ*) lesions[9] spread from the medial hepatic bile ducts to the hilar bile duct and the common bile duct, including their intramural glands (Figs. 5B and 5C). Chronic bile duct injury was shown around the carcinoma and BilIN lesions (Figs. 5B and 5D). No significant reactive changes of the hepatocytes were noted. Lymphatic, venous and perineural invasion, and lymph node metastasis were absent. Postoperatively, the patient was treated with radiation for the hepatic hilum (total 50 Gy), and thereafter has been receiving adjuvant chemotherapy (gemcitabine). The patient is doing well 30 months after the operation without recurrence.

Discussion

From an anatomical point of view, CC is classified as intrahepatic or extrahepatic, with the latter form being further divided into proximal or perihilar (Klatskin tumor) and distal, depending on the location of the cancer within the extrahepatic biliary system[1]. In our case, the cancer cells were spread widely from the intrahepatic bile duct to the right hepatic duct and common bile duct, which would be broadly classified as Type Ⅳ according to the Bismuth-Corlette classification of Klatskin tumors[10], but a curative operation was performed using an extended left hepatectomy with resection of the extrahepatic bile duct. Recent histological and molecular characterizations have highlighted the heterogeneity of CC, which may emerge in different sites of the biliary tree and with different macroscopic or morphologic features[11]. Moreover, histologically, flat-type "BilIN" and papillary-type "IPNB" were previously proposed to be precursors of invasive, perihilar intrahepatic CC[9]. These findings correspond with the different macroscopic appearance and histologic findings in our case. We suggested that our patient had experienced chronic bile duct injury due to an unknown cause, which might have induced the wide-spread BilIN lesions and IPNB with invasive carcinoma[11].

There are several established risk factors for CC, including parasitic infections, primary sclerosing cholangitis, biliary-duct cysts, hepatolithiasis, and toxins[2-5]. Other less-established potential risk factors include inflammatory bowel disease, hepatitis C virus infection, hepatitis B virus infection, cirrhosis, diabetes, obesity, alcohol, smoking, and host genetic polymorphisms[2,3,6]. In our case, a preoperative diagnosis of CC would have been difficult without cytology because the patient did not have any of the risk factors described above and was relatively young (39 years old). The typical age at presentation of CC is in the seventh decade of life[7,8]. In our case, the findings from cholangiography appeared to correspond to those of primary or secondary sclerosing cholangitis (pruned tree sign, multifocal stricture, and shaggy sign)[12], especially in the right intrahepatic bile duct where CC was absent. This suggests that CC might have been induced by secondary sclerosing cholangitis. However, our patient also did not have any of the risk factors for this disease, which include ascending cholangitis, oriental cholangiohepatitis, acquired immunodeficiency syndrome-related cholangitis, chemotherapy-induced cholangitis, ischemic cholangitis after liver transplantation, eosinophilic cholangitis, and metastasis[12].

In Japan, an epidemic of CC at the printing plant at which our patient worked was reported in a news release in May 2012. An investigation by the Ministry of Health, Labor and Welfare showed that 14 patients who worked in the factory had developed CC since 1996, and that seven had died of CC. Moreover, the mean age of the patients who died of CC was less than 40 years. Exposure to

suspected carcinogenic chemicals, including 1,2-dichloropropane, in the factory may have been one of the factors responsible for the carcinogenesis[13,14]. Currently, we cannot be certain that the CC in our patient was induced by carcinogenic chemicals, but the findings from cholangiography and the histological findings indicated possible chronic inflammation of the bile duct. Further epidemiological and environmental investigations are needed to establish risk factors for CC.

References

1. In: Nakamura Y, Minato H, Kida T, Terada T, editors. Pathology of cholangiocellular carcinoma. Tokyo: Springer-Verlag; 1992.
2. Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. Hepatology 2011;54:173-184.
3. Cardinale V, Semeraro R, Torrice A, Gatto M, Napoli C, Bragazzi MC, et al. Intra-hepatic and extra-hepatic cholangiocarcinoma: New insight into epidemiology and risk factors. World J Gastrointest Oncol 2010;2:407-416.
4. Kubo S, Kinoshita H, Hirohashi K, Hamba H. Hepatolithiasis associated with cholangiocarcinoma. World J Surg 1995;19:637-641.
5. Uenishi T, Hamba H, Takemura S, Oba K, Ogawa M, Yamamoto T, et al. Outcomes of hepatic resection for hepatolithiasis. Am J Surg 2009;198:199-202.
6. Yamamoto S, Kubo S, Hai S, Uenishi T, Yamamoto T, Shuto T, et al. Hepatitis C virus infection as a likely etiology of intrahepatic cholangiocarcinoma. Cancer Sci 2004;95:592-595.
7. Blechacz BR, Gores GJ. Cholangiocarcinoma. Clin Liver Dis 2008;12:131-150, ix.
8. Shaib Y, El-Serag HB. The epidemiology of cholangiocarcinoma. Semin Liver Dis 2004;24:115-125.
9. Nakanuma Y, Sasaki M, Sato Y, Ren X, Ikeda H, Harada K. Multistep carcinogenesis of perihilar cholangiocarcinoma arising in the intrahepatic large bile ducts. World J Hepatol 2009;1:35-42.
10. Bismuth H, Corlette MB. Intrahepatic cholangioenteric anastomosis in carcinoma of the hilus of the liver. Surg Gynecol Obstet 1975;140:170-178.
11. Cardinale V, Carpino G, Reid L, Gaudio E, Alvaro D. Multiple cells of origin in cholangiocarcinoma underlie biological, epidemiological and clinical heterogeneity. World J Gastrointest Oncol 2012;4:94-102.
12. Vitellas KM, Keogan MT, Freed KS, Enns RA, Spritzer CE, Baillie JM, et al. Radiologic manifestations of sclerosing cholangitis with emphasis on MR cholangiopancreatography. Radiographics 2000;20:959-975; quiz 1108-1109, 1112.
13. Guengerich FP, Kim DH, Iwasaki M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol 1991;4:168-179.
14. National Toxicology Program. NTP Toxicology and Carcinogenesis Studies of 1,2-Dichloropropane (Propylene Dichloride) (CAS No. 78-87-5) in F344/N Rats and B6C3F1 Mice (Gavage Studies). Natl Toxicol Program Tech Rep Ser 1986;263:1-182.

J Occup Health 2014; 56: 317–322

Journal of Occupational Health

Case Study

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

Shoji KUBO[1], Shigekazu TAKEMURA[1], Chikaharu SAKATA[1], Yorihisa URATA[1], Takayoshi NISHIOKA[1], Akinori NOZAWA[1], Masahiko KINOSHITA[1], Genya HAMANO[1], Yasuni NAKANUMA[2] and Ginji ENDO[3]

[1]Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, Japan, [2]Department of Human Pathology, Kanazawa University Graduate School of Medicine, Japan and [3]Department of Preventive Medicine and Environmental Health, Osaka City University Graduate School of Medicine, Japan

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.
(J Occup Health 2014; 56: 317–322)

Key words: Carbohydrate 19-9, Dilated bile ducts, γ-Glutamyl transpeptidase, Occupational cholangiocarcinoma, Organic solvent

Received Jan 20, 2014; Accepted Apr 11, 2014
Published online in J-STAGE Jun 21, 2013
Correspondence to: S. Kubo, Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan (e-mail: m7696493@msic.med.osaka-cu.ac.jp)

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).

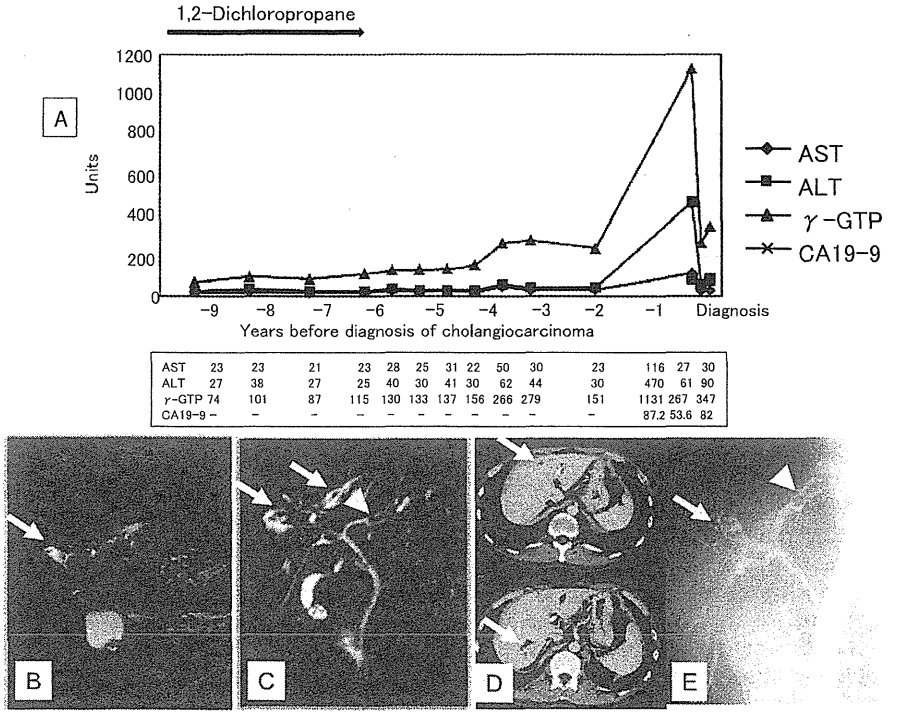

Fig. 1. Changes in laboratory test results and diagnostic imaging presentation before the diagnosis of cholangiocarcinoma in case 1. (A) Laboratory test results. (B) Magnetic resonance cholangiopancreatography (MRCP) 2 years before cholangiocarcinoma diagnosis. (C) MRCP 4 months before detection[2]. (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

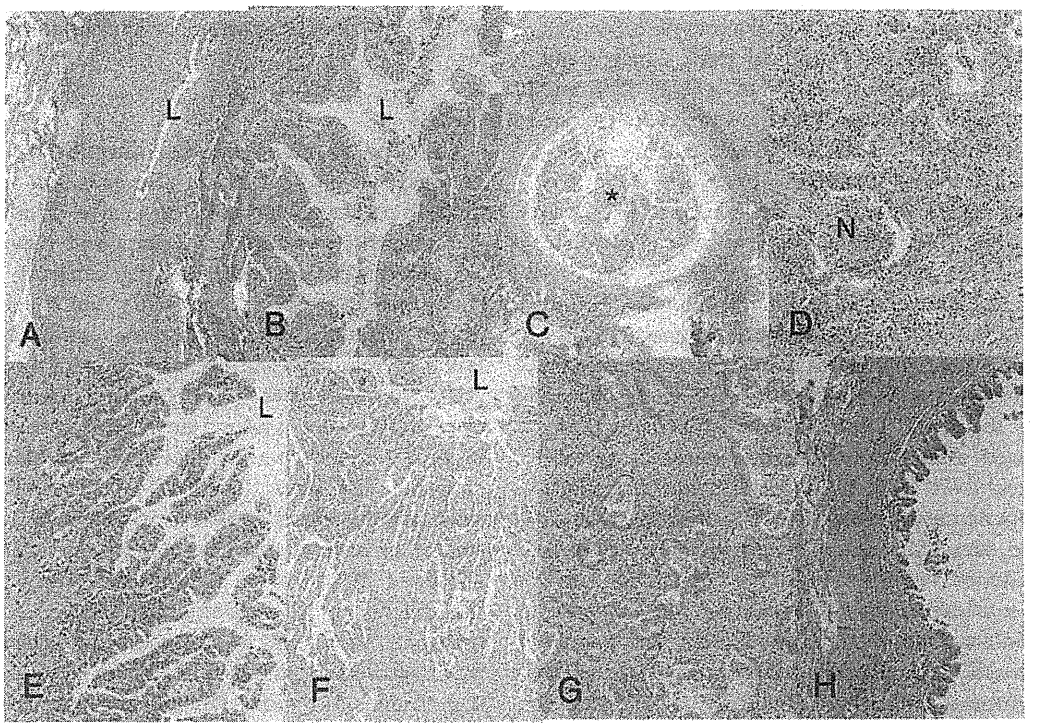

Fig. 2. Pathological findings of the resected specimens.
A, The large bile duct shows fibrous thichening of the duct wall and periductal tissue and erosion of the linig epithelia. L, bile duct lumen. HE. B, BilIN-2 lesion. L, Bile duct lumen. HE. C, The dilated bile duct contains neoplastic lining epithelia 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

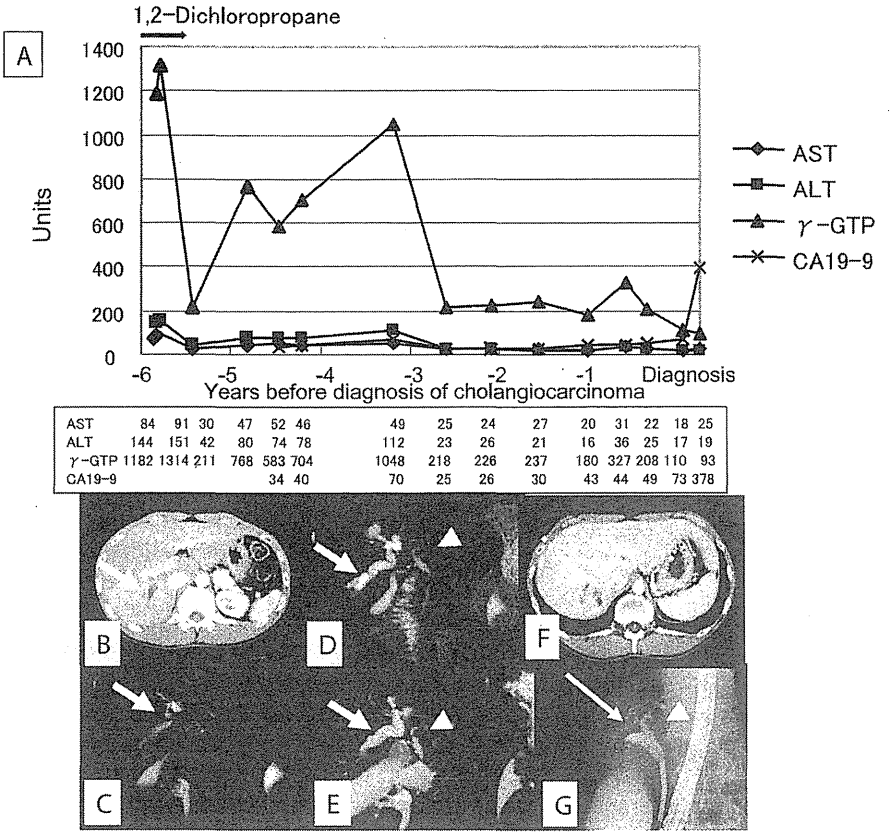

Fig. 3. Changes in laboratory test results and diagnostic imaging presentation before the diagnosis of cholangiocarcinoma in case 2. (A) Laboratory test results. (B) CT at 5 years before diagnosis. (C) MRCP at 3 years and 6 months before diagnosis. (D) MRCP at 8 months before diagnosis. (E) MRCP at 3 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.

Acknowledgments: This study was supported in part by Health and Labour Sciences Research Grants for Research on Occupational Safety and Health (the epidemiological and cause-investigated study of cholangiocarcinoma in workers of a printing company).

References

1) Kumagai S, Kurumatani N, Arimoto A, Ichihara G. Cholangiocarcinoma among offset colour proofprinting workers exposed to 1,2-dichloropropane and/or dichloromethane. Occup Environ Med 2013; 70: 508–10.
2) Kubo S, Nakanuma Y, Takemura S, et al. Case-series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan. J Hepato Biliary Pancreat Sci (in press).
3) National Institute of Occupational Safety and Health, Japan. Industrial accident in a printing company in Osaka [in Japanese]. Report A-2012-02, 2012. [Online]. 2012 [cited 2014 May 3]; URL: https://www.jniosh.go.jp/publication/pdf/houkoku.pdf
4) IARC (International Agency for Research on Cancer). List of classification by cancer sites with sufficient or limited evidence in humans, Volume 1 to 105. [Online]. 2012 [cited 2014 May 3]; URL: http://monographs.iarc.fr/ENG/Classification/index.php
5) Ministry of Health, Labour and Welfare: Occupational biliary tract cancer cases in Japan. [Online]. 2013 [cited 2014 May 3]; URL: http://www.mhlw.go.jp/english/policy/employ-labour/labour-standards/Occupational.html
6) Ikai I, Kudo M, Arii S, et al. Report of the 18th follow-up survey of primary liver cancer in Japan. Hepatology Res 2010; 40: 1043–59.
7) Zen Y, Adsay NV, Bardadin K, et al. Biliary intraepithelial neoplasia: an international interobserver agreement study and proposal for diagnostic criteria. Mod Pathol 2007; 20: 701–9.
8) International Labour Organization: International Chemical Safety Cards. [Online]. 1999 [cited 2014 May 3]; URL: http://www.ilo.org/dyn/icsc/showcard.display?p_lang=en&p_card_id=0441
9) Vitellas KM, Keigan MT, Freed KS, et al. Radiologic manifestations of sclerosing cholangitis with emphasis on MR cholangiopancreatography. Radio Graphics 2000; 20: 959–75.
10) Itatsu K, Zen Y, Ohira S, et al. Immunohistochemical analysis of the progression of flat and papillary preneoplastic lesions in intrahepatic cholangiocarcinogenesis in hepatolithiasis. Liver International 2007; 27: 1174–84.
11) Chen TC, Nakanuma Y, Zen Y, et al. Intraductal papillary neoplasia of the liver associated with hepatolithiasis. Hepatology 2001; 34: 651–8.
12) Watanapa P, Watanapa WB. Liver fluke-associated cholangiocarcinoma. Br J Surg 2002; 89: 962–70.
13) Ikai I, Kudo M, Arii S, et al. Report of the 18th follow-up survey of primary liver cancer in Japan. Hepatology Res 2010; 40: 1043–59.
14) Miyakawa S, Ishihawa S, Horiguchi A, Takada T, Miyazaki M, Nagakawa T. Biliary tract cancer treatment: 5,584 result from the biliary tract cancer statistics registry from 1998 to 2004 in Japan. J Hepatobiliary Pancreat Surg 2009; 16: 1–7.

J Occup Health 2015; 57: 87–90

Journal of Occupational Health

Case Study

Severe acute hepatitis in a printing company worker: A case study

Shoji KUBO[1], Koichi MATSUZAKI[2], Toshihito SEKI[2], Masahiko OHSAWA[3], Shinji KUMAGAI[4] and Ginji ENDO[5]

[1]Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, Japan, [2]Department of Gastroenterology and Hepatology, Kansai Medical University, Japan, [3]Department of Diagnostic Pathology, Osaka City University Graduate School of Medicine, Japan, [4]Department of Management of Occupational Safety and Health, University of Occupational and Environmental Health, Japan and [5]Department of Preventive Medicine and Environmental Health, Osaka City University Graduate School of Medicine, Japan

Abstract: Severe acute hepatitis in a printing company worker: A case study: Shoji KUBO, *et al.* Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine—Objectives: It has been reported that chlorinated organic solvent is a cause of hepatitis. **Methods:** we investigate clinical and pathological findings of a patient with severe acute hepatitis who was exposed to chlorinated organic solvents. **Results:** A 34-year-old man who was exposed to chlorinated organic solvents including dichloromethane, 1,2-dichloropropane, and trichloroethylene, presented with general fatigue, vomiting, and diarrhea. At admission, his laboratory test results showed extremely elevated aspartate aminotransferase (4,872 IU/l), alanine aminotransferase (3,000 IU/l), and lactate dehydrogenase (11,600 IU/l) levels and a prothrombin level below normal (41%). No encephalopathy was noted. These findings were indicative of severe acute hepatitis. Viral hepatitis, autoimmune hepatitis, alcoholic disease, bile duct disease, and viral infection were excluded as causes of hepatitis by clinical, laboratory, and imaging findings. After diagnosis, the patient was administered fresh frozen plasma and glucagon-insulin therapy. Liver function recovered within a few weeks, and a liver biopsy performed 25 days after admission showed the recovery phase after acute liver damage. **Conclusions:** These clinical and pathological findings indicate that exposure to chlorinated organic solvents may have induced severe acute hepatitis in this patient.
(J Occup Health 2015; 57: 87–90)

Received Aug 18, 2014; Accepted Oct 14, 2014
Published online in J-STAGE Nov 19, 2014
Correspondence to: S. Kubo, Department of Hepato-Biliary-Pancreatic Surgery, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abeno-ku, Osaka 545-8585, Japan (e-mail: m7696493@msic.med.osaka-cu.ac.jp)

Key words: 1,2-Dichloropropane, Printing company, Severe acute hepatitis, Trichloroethylene

The toxicities of various chemicals, including organic solvents, have been reported, and methods for protection and occupational exposure limits have been established. Most organic solvents are mainly absorbed into the body via inhalation, ingestion, and the skin, and they often directly affect the eyes, skin, and respiratory tract. Absorbed organic solvents are toxic to the nervous system, liver, kidney, and heart[1, 2]. Hepatic damage after exposure to certain organic solvents has been described[3–9]. Here, we report a case of severe acute hepatitis in a printing company worker who was exposed to various chemicals, including organic solvents such as 1,2-dichloropropane (DCP), dichloromethane (DCM), and trichloroethylene (TCE). This study was performed according to the Declaration of Helsinki (2008), and the patient provided written informed consent.

Case Report

The patient started work in an offset color proof-printing department of a company in 1986. The present building was constructed in 1991. The printing room was located in the first basement floor of the building, with a front room adjacent to the printing room. The ventilation rates of these rooms were very low because of the basement location and the low capacity of the installed ventilation equipment. The patient made printing plates in the front room. In the process, he used high-purity TCE to remove stains from glass plates for about one year just before developing severe acute hepatitis. The amount of TCE he used per day was estimated to be 1-2 l based on his memory. Because no respiratory protection was

provided, he have been exposed to high levels of TCE.

In the printing room, proof-printing workers used large amount of organic solvent cleaner to remove ink residue from a rubber transcription roller. The cleaners was a mixture of gasoline (50% by weight) and 1,1,1-trichloroethane (50%) before 1989; it was a mixture of DCP (50–60%), DCM (15–25%), and 1,1,1-trichloroethane (15–25%) from approximately 1985 to 1992-1993; and it was a mixture of DCP (40–50%), DCM (40–50%) and petroleum hydrocarbons (1–10%) from 1992–1993 to March 1996; and it was nearly pure DCP solvent (98%) from April 1996 to October 2006. Airborne solvent concentrations in the printing room were estimated to be extremely high, which was confirmed in an experiment conducted by the Japanese National Institute of Occupational Safety and Health[10].

Furthermore, because the contaminated air of the printing room flowed into the front room due to positive pressure in the printing room, the airborne solvent concentrations were also estimated to have been high in the front room. Consequently, the patient was also exposed to these chemicals when working in the front room. In addition to making printing plates, he also supervised the progress of printing mainly in the front room but frequently went into the printing room to provide guidance and occasionally to conduct proof-printing. When working in the printing room, he was exposed to high levels of the abovementioned chlorinated organic solvents. Other chemicals such as kerosene and inks were also used in the department.

The patient (at 34 years of age) experienced general fatigue, vomiting, and diarrhea and visited a hospital in December 1996. According to the period of solvent use, he had been exposed to DCP and TCE just before the onset of symptoms and to DCM within 1 year before onset. He had drank 350 m*l* of beer per day during previous 10 years (<80 g of ethanol daily, which is the lower limit for alcoholic liver disease[11]) and smoked 20 cigarettes/day during the previous 14 years. He had no history of blood transfusion, sometimes took vitamins and aspirin for headaches, and had a body mass index of 18.3.

At admission, the patient was lucid with no abnormal neurological system or respiratory tract findings. The liver was palpable in the right infracostal region at a two finger widths. No dermatitis was noted. The laboratory test results at admission are shown in Table 1. The aspartate aminotransferase (AST, 4,872 IU/l), alanine aminotransferase (ALT, 3,000 IU/l); and lactate dehydrogenase (LDH, 11,160 IU/l) levels were markedly elevated, and the prothrombin test value was 41%. The concentrations of total bilirubin and direct bilirubin were 1.2 mg/d*l* and 0.2 mg/d*l*, respectively.

Table 1. Laboratory test results at admission

Red blood cell ($\times 10^4$/mm^3)	448
Hemoglobin (g/d*l*)	13.6
White blood cell (/mm^3)	6,800
Prothrombin test (%)	41
Aspartate aminotransferase (U/*l*)	4,872
Alanine aminotransferase (U/*l*)	3,000
Alkaline phosphatase (U/*l*)	140
Total bilirubin (mg/d*l*)	1.2
Direct bilirubin (mg/d*l*)	0.2
Lactate dehydrogenase (U/*l*)	11,160
γ-Glutamyl transpeptidase[a] (U/*l*)	45
BUN[a] (mg/d*l*)	18
Creatine[a] (mg/d*l*)	0.6
Na[a] (mEq/m*l*)	137
K[a] (mEq/m*l*)	3.6
Cl[a] (mEq/m*l*)	105
CRP (mg/d*l*)	1.9

[a]Tests performed the day after admission.

The serum alkaline phosphatase and γ-glutamyl transpeptidase (γ-GTP) levels were within the reference range. Eosinophilia was not detected. The results for hepatitis viral markers (IgM-HA antibody, hepatitis B e antigen and antibody, hepatitis B surface antigen and antibody, hepatitis B core antibody, hepatitis B virus DNA polymerase, hepatitis C virus [HCV] antibody, HCV RNA, hepatitis D virus antibody, and GBV-C RNA) were negative. The patient was positive for cytomegalovirus IgG and Epstein-Barr (EB) virus IgG antibody, but negative for cytomegalovirus IgM antibody and EB virus IgM antibody, indicating previous infections with cytomegalovirus and EB virus. Serum anti-nuclear antibody and lupus erythematosus (LE) test results were negative. Serum complement (CH50; 50% hemolytic unit of complement), carcinoembryonic antigen, and carbohydrate antigen 19–9 levels were within the reference ranges. Ultrasonography and computed tomography showed mild hepatomegaly but no abnormal findings in the biliary system. From these findings, severe acute hepatitis was diagnosed, and the patient was treated with fresh frozen plasma and glucagon-insulin therapy. Liver function recovered within a few weeks (Fig. 1). A liver biopsy performed 25 days after admission showed size inequality of hepatocytes and multinuclear hepatocytes (Fig. 2). Many phagocytes were present in the hepatic lobules, and mild lymphocytes infiltration and fatty droplets in a few hepatocytes were seen. Fibrous expansion of portal areas and cholestasis were not observed. These findings are indicative of the recovery phase after acute liver damage. The

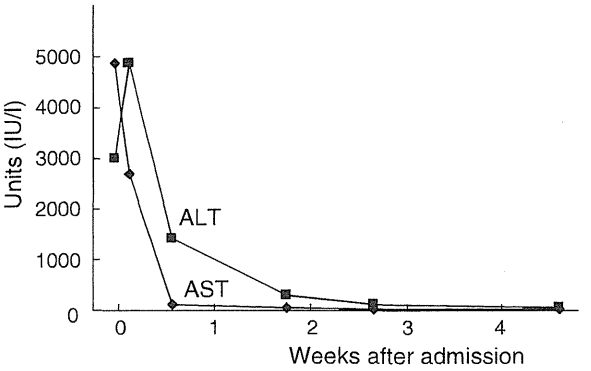

Fig. 1. Changes in alanine and aspartate aminotransferase levels after admission.

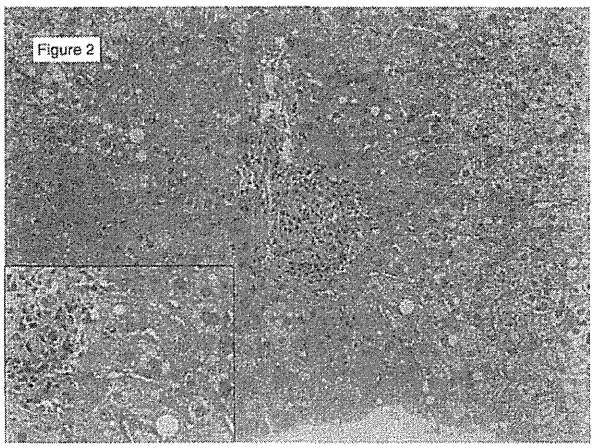

Fig. 2. Pathological findings of liver biopsy (hematoxylin and eosin, 20×, and inset, 40×).

clinical course and pathological findings indicate that the patient's severe acute hepatitis was not caused by viral hepatitis, autoimmune hepatitis, alcoholic disease, bile duct disease, or viral infection (cytomegalovirus and EB virus) but instead was caused by exposure to chlorinated organic solvents.

Use of TCE was stopped at the printing company after this event. Since his discharge from the hospital, he has not been exposed to high concentrations of chlorinated organic solvents. The patient is now in good health.

Discussion

Severe acute hepatitis developed in a worker in an offset color proof-printing department of a company. The three criteria for the diagnosis of toxic hepatitis include the following: (1) liver damage after occupational exposure to a substance, considering the patient's work history and current workplace; (2) elevated liver enzyme activity to at least double the upper limit of the reference range; and (3) exclusion of tertiary conditions such as other causes of liver damage[12, 13]. The patient in this study was exposed to various solvents, including DCP, DCM, and TCE. His serum AST, ALT and LDH levels were remarkably elevated at the time of admission to the hospital and improved rapidly after admission (stopping exposure) and treatment. The patient did not have any known cause of severe acute hepatitis, such as viral hepatitis, autoimmune hepatitis, alcoholic liver disease, viral infection (adenovirus, cytomegalovirus, or EB virus), or biliary tract disease.

Acute toxicity cause by DCP, DCM and TCE has been reported by several investigators[3–9]. The International Chemical Safety Cards produced of the International Labour Organization[2] warn that long-term or repeated exposure to DCM or DCP may affect the liver and kidneys. Repeated or prolonged contact of skin with TCE may cause dermatitis. This solvent may affect the central nervous system, liver, and kidneys. Recently, Chang *et al.*[14] reported that exposure to both lead and organic solvents is dangerous, even if exposure to each of the individual components is within the respective permissible limit. In the present case, the patient developed symptoms during exposure to DCP and TCE and within 1 year of exposure to DCM. There were few workers exposed to both DCP and TCE. Therefore, DCP and TCE were suspected to be the causative agents of the severe acute hepatitis in the patient. DCM also might have contributed to the development of hepatitis. In addition, mixed exposure to such organic solvents might synergize towards the development of the hepatitis.

Toxic hepatitis after exposure to chemicals can be divided into three types: hepatocellular, cholestatic, and mixed type[15]. Laboratory test results in this patient showed elevated AST, ALT, and LDH levels and a decreased prothrombin value, whereas the serum alkaline phosphatase and γ-GTP levels were within the reference ranges. These results indicate that the hepatitis in this patient should be classified into as a hepatocellular type. Recently, an outbreak of cholangiocarcinoma occurred in this same company, and chlorinated organic solvents, particularly DCM and DCP, were suspected to play a causative role[16, 17]. In patients with occupational cholangiocarcinoma, laboratory test results showed elevated γ-GTP levels (with or without elevated AST and/or ALT levels), and pathological findings demonstrated chronic bile duct injury and non-injured hepatocytes[17]. In addition, the patients with occupational cholangiocarcinoma were not exposed to TCE. Therefore, the mechanism causing severe acute hepatitis in the present patient seemed to be different from that causing occupational cholangiocarcinoma.

Although this patient's liver function improved rapidly after restricting further exposure and administering fresh frozen plasma and glucagon-insulin therapy, death due to acute liver failure was reported in a patient with suspected TCE exposure[9]. Thus, regular assessment of liver function in workers exposed to such chlorinated organic solvents is important because excessive exposure may induce lethal acute hepatitis.

References

1) Baker EL. A review of recent research on health effects of human occupational exposure to organic solvents: a critical review. J Occup Med 1994; 26: 1079–92.
2) International Labour Organization. International Chemical Safety Cards (ICSC). http://www.ilo.org/dyn/icsc/showcard.home
3) Pozzi C, Marai P, Pnti R, et al. Toxicity in man due to stain removers containing 1,2-dichloropropane. Br J Indust Med 1985; 42: 770–2.
4) Mizutani K, Shinomiya K, Shinomiya T. Hepatotoxicity of dichloromethane. Forensci Sci Int 1988; 38: 113–28.
5) Sakai T, Miyake K, Kurata M, et al. A case of severe hepatic microcirculatory failure with jaundice and fibrosis around the sinusoid caused by long-term exposure of methylenechloride. Jpn J Gastroenterol 1991; 88: 185–9 (in Japanese with English abstract).
6) Chang YL, Yang CC, Deng JF, et al. Diverse manifestations of oral methylene chloride poisoning: report of 6 cases. J Toxicol Clin Toxicol 1999; 37: 487–504.
7) Chae HJ, Lee SK, Kim JY, Lee SC, Shin DH, Moon JD. Exforative dermatitis and toxic hepatitis associated with occupational exposure to trichloroethylene. Korean J Occup Environ Med 2003; 15: 111–7.
8) Anagnostopoulos G, Sakorafas GH, Grigoriadis K, et al. Hepatitis caused by occupational exposure to trichloroethylene. Acta Gastroenterol Belg 2004 67: 355–7.
9) Takaki A, Suzuki H, Iwasaki Y, et al. A 27-year-old man who died of acute liver failure probably due to trichloromethylene abuse. J Gastroenterol 2008; 43: 239–42.
10) National Institute of Occupational Safety and Health, Japan. Industrial accident in a printing company in Osaka [in Japanese]. Report A-2012-02, 2012. [Online]. 2012 [cited 2014 Sept 28]: Available from URL: http://www.mhlw.go.jp/stf/houdou/2r9852000002ioeh-att/2r9852000002ioik.pdf
11) Lelbach WK. Cirrhosis in the alcoholic and its relation to the volume of alcohol abuse. Ann N Y Acad Sci 1975; 252: 85–105.
12) Benichou C, Danan G, Fkahault A. Causality assessment of adverse reactions to drugs-II. An original model for validation of drug causality assessment menthods: case reports with positive rechanllenge. J Clin Epidemiol 1993; 46: 1331–6.
13) Maria VA, Victorino RM. Development and validation of a clinical scale for the diagnosis of drug-induced hepatitis. Hepatology 1997; 26: 664–9.
14) Chang WJ, Joe KT, Park HY, Jeong JD, Lee DH. The relationship of liver function tests to mixed exposure to lead and organic solvents. Ann Occup Environ Med 2013; 25: 5. [Online]. 2013 [cited 2014 Sept 28] : Available from URL: http://www.aoemj.com/content/25/1/5
15) Malaguarnera G, Cataudella E, Giordano M, Nunnari G, Chisari G, Malaguarnera M. Toxic hepatitis in occupational exposure to solvents. World J Gastroenterol 2012; 28: 2756–66.
16) Kumagai S, Kurumatani N, Arimoto A, Ichihara G. Cholagiocarcinoma among offset colour proof-printing workers exposed to 1,2-dichloropropane and/or dichloromethane. Occup Environ Med 2013; 70: 508–10.
17) Kubo S, Nakanuma Y, Takemura S, et al. Case-series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan. J HepatoBiliary Pancreat Sci 2014; 21: 479–88.