

Time Course of Blood Parameters in Printing Workers with Cholangiocarcinoma

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Abstract: Time Course of Blood Parameters in Printing Workers with Cholangiocarcinoma: Shinji KUMAGAI, *et al.* Department of Occupational and Environmental Management, University of Occupational and Environmental Health, Japan—**Objectives:**

We previously reported a cluster of cholangiocarcinoma patients among proof-printing workers who were exposed to 1,2-DCP for a long term. The present study was conducted to evaluate blood parameters in these proof-printing workers during and after exposure.

Methods: Health examination records during employment and after retirement were obtained for ten cholangiocarcinoma patients to analyze their blood parameters. The patients and/or their relatives were also interviewed about lifestyle and occupational history.

Results: All study patients were exposed to 1,2-DCP for 6–17 years. Red blood cells, hemoglobin, hematocrit, total cholesterol, triglycerides, and fasting plasma glucose were within the standard ranges for almost all patients, but the γ -glutamyl transpeptidase (γ -GTP) levels exceeded the standard range during 1,2-DCP exposure for six patients. Two of the six patients were diagnosed with cholangiocarcinoma during 1,2-DCP exposure, and the other four patients were diagnosed 1–9 years after termination of exposure. The remaining four patients had γ -GTP levels within the standard range during 1,2-DCP exposure, but had increased γ -GTP levels thereafter, and were diagnosed with cholangiocarcinoma 4–10 years after termination of exposure. Aspartate aminotransferase and alanine aminotransferase levels started to increase following the increase in γ -GTP levels. **Conclusions:** Workers exposed to 1,2-

DCP should be provided with periodic health examinations during and after exposure. In the examination, even small increases in γ -GTP levels should be considered a signal of early development of cholangiocarcinoma.

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Key words: 1,2-Dichloropropane, Cholangiocarcinoma, Dichloromethane, Printing worker, Serum liver enzymes

We previously reported a cluster of 11 cholangiocarcinoma patients among proof-printing workers employed at a printing company in Osaka, Japan¹. The proof-printing room was located in the first basement of the current company building and had seven single-color printing machines. There was little ventilation due to the basement location and low capacity of the installed ventilation equipment. When changing colors, the printing workers frequently used highly volatile organic solvents including 1,2-dichloropropane (1,2-DCP) and/or dichloromethane (DCM) to remove ink from the ink-transcription rubber roller (called the “blanket”) and kerosene to remove ink from the ink-roller. They wore plastic gloves during ink removal but did not use any respiratory protection while working. We estimated exposure concentrations to be 100–670 ppm for 1,2-DCP and 80–540 ppm for DCM¹ based on a reproductive experiment conducted by the Japan National Institute of Occupational Safety and Health².

Thereafter, other cholangiocarcinoma patients appeared among former or current workers of the company, and the total number of patients increased to 17 as of June 2013³. Kubo *et al.* reported in their case series⁴ that all of the 17 patients were exposed to 1,2-DCP, 11 of the patients were also exposed to DCM, and 8 of the patients were also exposed to

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1,1,1-TCE. Kubo *et al.*⁴⁾ also reported that many other chemicals had been used in the printing department (dichlorofluoroethane, 2-butanol, 2-methylpentane, 3-methylpentane, n-hexane, cyclohexane, isopropyl alcohol, ethanol, diethylene glycol monobutyl ether, propylene glycol monomethyl ether, 2-methyl-2,4-pentadiol, 3-methyl-3-methoxybutanol, solvent naphtha, xylene, mineral oil, hydrocarbons, aromatic hydrocarbons and inks), but these chemicals were ruled out as possible causative agents because of their low amount used and/or short period of exposure.

These findings suggest that 1,2-DCP might play an important role in the development of cholangiocarcinoma. Consequently, it would be interesting to investigate how liver function varied during and after exposure to 1,2-DCP. In this study, we evaluated blood parameters, with a special focus on serum liver enzymes, in cholangiocarcinoma patients during and after 1,2-DCP exposure. This study was approved by the Ethics Committee of the University of Occupational and Environmental Health.

Subjects and Methods

Medical informations

Company records of periodic annual health examinations conducted from 1999 until retirement were still available for 13 of the 17 cholangiocarcinoma patients. Blood test records from health examinations included levels of serum liver enzymes (aspartate aminotransferase [AST], alanine aminotransferase [ALT], γ -glutamyl transpeptidase [γ -GTP]) and parameters of hematology (red blood cells [RBC], hemoglobin [Hb], hematocrit [Ht]), lipid metabolism (total cholesterol [T-Chol], triglycerides [TG]) and glucose metabolism (fasting plasma glucose [FPG]).

Ten of the 13 patients gave consent to participate in this study and provided the above records. Four of these patients (B, D, G, J) also provided records of health examinations conducted after retirement from the printing company. In addition, all of the 10 patients provided medical information (hematological, imaging and pathological findings and medical and/or death certificates) obtained from their hospitals through right-to-know requests. Blood parameters were evaluated by comparing values with standard ranges used by the facility that had conducted the periodic health examinations.

Occupational history and lifestyle

To obtain information regarding their occupational history and lifestyle, we conducted detailed interviews with seven patients (A, B, E, G, H, I, J) and the relatives of three deceased patients (C, D, F) and confirmed the reliability of the obtained information by comparing it with written information (e.g.,

pension records, medical records). To identify the chemicals used, we referred to information related to chemicals obtained from the printing company and conducted detailed interviews with more than 40 current and former printing workers. Chemical components were identified based on certificates provided by the companies that sold them.

Results

Patient characteristics

Patient characteristics are shown in Table 1. All patients were male. One patient drank 40 g of alcohol almost every day, and another patient drank 100 g of alcohol three or four days a week. Seven patients were smokers with a Brinkman index of 50–380 cigarettes/day \times years. According to the medical records, tests for hepatitis B surface antigen and hepatitis C virus antibody were negative for all patients. None of the patients had primary sclerosing cholangitis, liver fluke infestation, biliary stones or fibropolycystic liver disease.

All patients had worked in offset color proof-printing at the company for 6–19 years (mean, 12 years). Figure 1 shows the time course of exposure to chemicals for all 10 patients. They were exposed to 1,2-DCP for 6–17 years (mean, 10 years) and kerosene for 6–19 years (mean, 12 years). Five patients were also exposed to DCM for 2–8 years (mean, 5 years), and three patients were additionally exposed to 1,1,1-trichloroethane for 3–4 years (mean, 3 years). In addition, three patients had recently been exposed to glycol ethers, alcohols and/or cycloaliphatic hydrocarbons for 6–7 years (mean, 7 years). The study patients were diagnosed with cholangiocarcinoma 7–20 years (mean, 14 years) after their first exposure to 1,2-DCP (black arrows, Fig. 1), and the age at diagnosis was 25–40 years (mean, 35 years).

Blood parameter levels

Figure 1 also shows the time course of serum liver enzyme levels for each patient. In Patient F, the γ -GTP levels exceeded the standard range (<86 IU/l) four years after the first exposure to 1,2-DCP and reached 1,730 IU/l three years later; he was diagnosed with cholangiocarcinoma seven years after the first exposure to 1,2-DCP. In Patient C, a similar time course of γ -GTP levels was observed, although he was exposed to not only 1,2-DCP but also DCM for the first seven years and additionally to 1,1,1-TCE for the first three years.

In Patient G, the γ -GTP levels increased to 83 IU/l, which is within the standard range, during exposure to 1,2-DCP. Thereafter, he quit the printing company, but the γ -GTP levels increased to more than 200 IU/l; he was diagnosed with cholangiocarcinoma fifteen

Table 1. Characteristics of 10 cholangiocarcinoma patients including baseline statistics and cancer data

Patient	Birth year	Drinking		Smoking (cigarettes/day × years)	Employment duration	Year of diagnosis	Year of death
		Frequency (days/week)	Alcohol (g/day)				
A	1969	<1	—	300	1988–1999 [11]	2007 [19] (38)	Alive
B	1969	<1	—	380	1989–1992 1994–2000 [9]	2009 [20] (40)	2013 (43)
C	1969	<1	—	Nonsmoker	1989–2006 [17]	2004 [15] (35)	2006 (37)
D	1969	2–3	15	230	1994–2004 [10]	2009 [15] (39)	2010 (40)
E	1967	5<	40	180	1994–2013 [19]	2007 [13] (40)	Alive
F	1978	3–4	100	160	1996–2005 [9]	2003 [7] (25)	2005 (27)
G	1978	2–3	70	Nonsmoker	1997–2003 [6]	2012 [16] (34)	Alive
H	1978	<1	—	50	1997–2012 [15]	2010 [13] (31)	Alive
I	1971	<1	—	Nonsmoker	1999–2013 [14]	2010 [11] (39)	Alive
J	1981	<1	—	160	2000–2006 [6]	2012 [12] (31)	Alive
Mean	1973				[12]	[14] (35)	(37)

[]: Employment duration in years, [_]: Interval in years from the first employment, (): Age in years.

years after the first exposure. In Patient B, a similar time course of γ -GTP levels was observed, although he was exposed to not only 1,2-DCP but also DCM and 1,1,1-TCE.

In Patient J, the γ -GTP levels exceeded the standard range five years after the first exposure to 1,2-DCP and reached 1,310 IU// one year later. He then quit the printing company, and the γ -GTP levels rapidly decreased to 211 IU//. However, the γ -GTP levels increased again to 797 IU// one year after termination of exposure. He then began medical treatment with ursodeoxycholic acid, and the γ -GTP levels decreased to about 200 IU//. Nevertheless, he was diagnosed with cholangiocarcinoma twelve years after the first exposure.

In Patient I, the γ -GTP levels exceeded the standard range during exposure to 1,2-DCP. Thereafter, although he used glycol ethers, alcohols and cycloaliphatic hydrocarbons instead of 1,2-DCP, the γ -GTP levels continued to be high, and he was diagnosed with cholangiocarcinoma eleven years after the first exposure to 1,2-DCP. In Patient E, a similar time course of γ -GTP levels was observed, although he was also exposed to DCM for the first two years.

In Patient D, the γ -GTP levels did not increase during exposure to 1,2-DCP, but they did increase to 78 IU//, which is within the standard range, one year after termination of exposure and reached 1,980 IU// fifteen years after the first exposure to 1,2-DCP; he was then diagnosed with cholangiocarcinoma. In Patient A, a similar time course of γ -GTP levels was observed.

In Patient H, the γ -GTP levels did not increase during exposure to 1,2-DCP. After 1,2-DCP exposure, he was exposed to glycol ethers, alcohols and cycloaliphatic hydrocarbons for six years. The γ -GTP levels exceeded the standard range two years after termination of 1,2-DCP exposure (11 years after the first exposure) and reached 1,200 IU// thirteen years after the first exposure, and he was diagnosed with cholangiocarcinoma.

The AST levels in eight patients and ALT levels in nine patients exceeded the standard range just before cholangiocarcinoma diagnosis, but the levels were less than the γ -GTP levels (Fig. 1). The RBC, Hb, Ht, T-Cho, TG and FPG levels were within the standard range, except for T-Cho in two patients, in the last annual health examination conducted during expo-

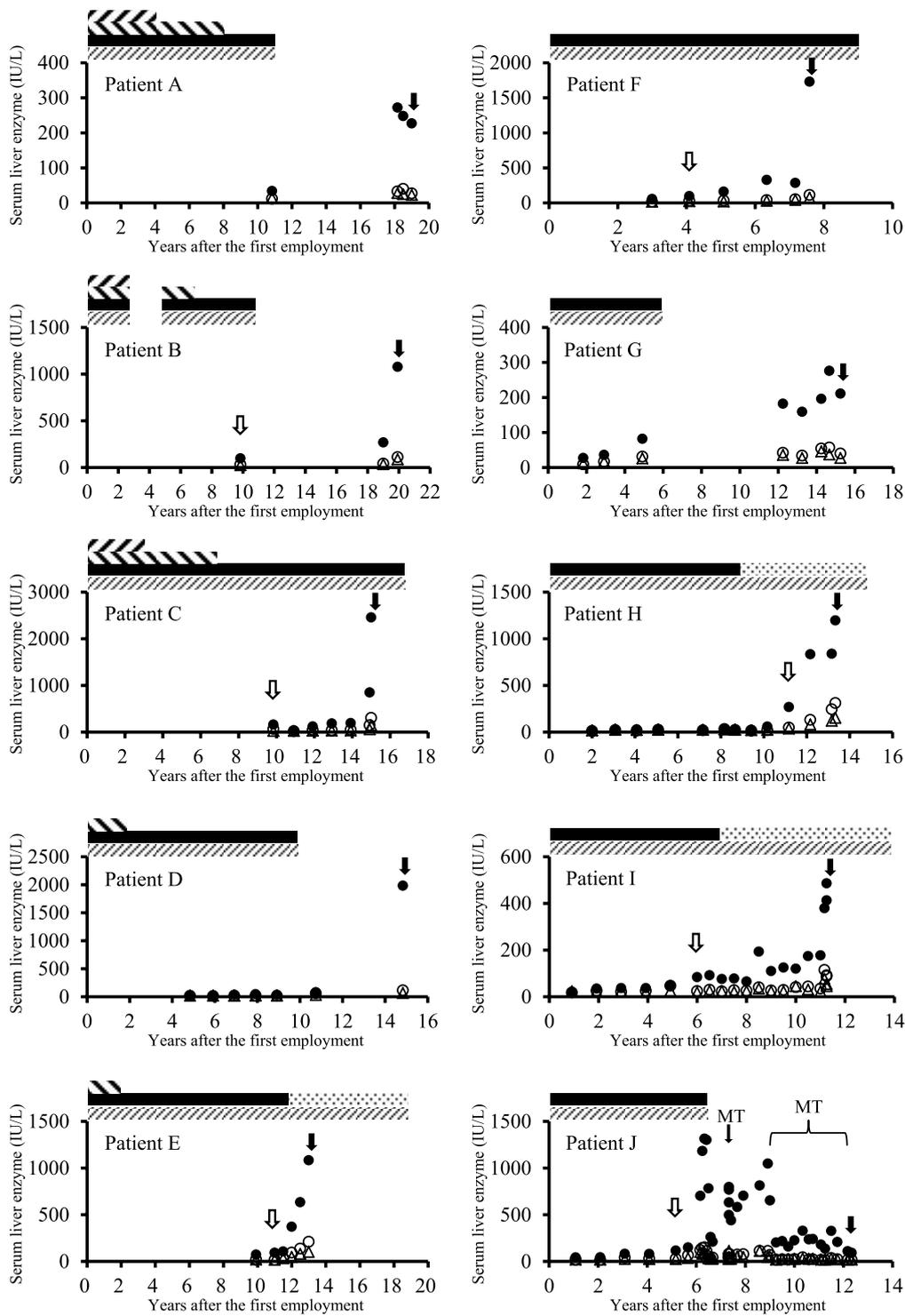


Fig. 1. Time course of serum liver enzyme levels for ten cholangiocarcinoma patients.
 \triangle : aspartate aminotransferase (AST), \circ : alanine aminotransferase (ALT), \bullet : γ -glutamyl transpeptidase (γ -GTP), \blacksquare : 1,2-dichloropropane, \diagup : dichloromethane, \diagdown : 1,1,1-trichloroethane, \dots : glycol ethers, alcohols and cycloaliphatic hydrocarbons, \diagup : kerosen, White arrow: γ -GTP>standard range, Black bold arrow: diagnosis, MT: medical treatment.

sure to 1,2-DCP for eight patients, and in the annual health examination conducted just before diagnosis of cholangiocarcinoma (during exposure to 1,2-DCP) for Patients C and F (data are not shown).

Discussion

As stated in the Introduction, Kubo *et al.* reported that all 17 patients who worked at the Osaka printing company had been exposed to 1,2-DCP for a long term and that chemicals used other than chlorinated organic solvents were ruled out as possible causative agents because of their low amount used and/or short period of exposure. This study reconfirmed that all ten of our patients had been exposed to 1,2-DCP for a long term and found that they did not have any known risk factors of cholangiocarcinoma including primary sclerosing cholangitis, liver fluke infestation, biliary stones, fibropolycystic liver disease, viral hepatitis, exposure to thorotrast, and heavy drinking and smoking⁵. These findings suggest that 1,2-DCP might play an important role in the development of cholangiocarcinoma. Consequently, hereafter, based on the hypothetical premise of such a causal relationship, we tried to interpret the time course of blood parameters in our ten patients because this premise would be seem to be safer for preventing development of cholangiocarcinoma in workers exposed to 1,2-DCP.

As shown in Fig. 1, in Patients C and F, the γ -GTP levels increased and cholangiocarcinoma was diagnosed during 1,2-DCP exposure. In Patient J, the γ -GTP levels increased during 1,2-DCP exposure and decreased after termination of exposure, but they later increased again; he was then diagnosed with cholangiocarcinoma. These findings lead to the following two hypotheses: 1) 1,2-DCP caused the increase in γ -GTP levels and cholangiocarcinoma independently, and 2) 1,2-DCP initially caused precancerous or early cancerous lesions in various sites of the bile ducts, which led to the increase in γ -GTP levels, and subsequently the lesions developed into cancer large enough to be detectable.

In Patient D, the γ -GTP levels did not increase during exposure to 1,2-DCP but began to increase after termination of exposure; he was then diagnosed with cholangiocarcinoma. In this case, 1,2-DCP did not directly cause the increase in γ -GTP levels, suggesting that the second hypothesis may be applicable. Kubo *et al.* reported that precancerous or early cancerous lesions, such as biliary intraepithelial neoplasia and intraductal papillary neoplasm, as well as nonspecific bile duct injuries, such as fibrosis, were observed in various sites of the bile ducts and peribiliary glands, particularly in the large and hilar bile ducts in all eight of the 17 patients whose operative specimens were available⁴. These findings are

not contradictory to our hypothesis.

Patients A and H showed no increase in γ -GTP levels during exposure to 1,2-DCP; however, the levels exceeded the standard range after termination of exposure, and both of these patients were diagnosed with cholangiocarcinoma. Although they were exposed to other organic solvents during 1,2-DCP exposure or after termination of 1,2-DCP exposure, cholangiocarcinoma was likely due to exposure to 1,2-DCP, as the time course was similar to that in Patient D. In other patients (B, E, G, I), the time course of γ -GTP levels and diagnosis can be explained by the above hypothesis. However, we cannot deny the possibility that 1,2-DCP could directly cause an increase in serum liver enzyme levels, because hepatic damage has been observed in acute poisoning cases⁶.

In our patients, the γ -GTP levels exceeded the standard range 4–11 years after the first exposure to 1,2-DCP, and they were diagnosed with cancer 2–10 years after the increase in γ -GTP levels. The AST and ALT levels began to increase following the increase in γ -GTP levels. These findings demonstrate that the increase in γ -GTP levels may be an important biomarker for predicting the early development of cholangiocarcinoma in workers exposed to 1,2-DCP. To prevent this cancer in 1,2-DCP exposed workers, when an increase in γ -GTP levels is observed in a health examination, we should not assume that the increase is due to alcohol intake but rather suspect cholangiocarcinoma, even if the increase is small.

This study also found that patients who had levels of serum liver enzymes within the standard range during 1,2-DCP exposure were diagnosed with cholangiocarcinoma 4–10 years after termination of exposure, which suggests that pre-cancerous or early-cancerous lesions began to develop in bile duct epithelial cells even when the increase in γ -GTP levels was not detected. Accordingly, 1,2-DCP exposed workers should be provided with periodic health examinations, in particular examinations to assess serum liver enzymes, during exposure as well as after termination of exposure. In examinations, serum tumor markers (e.g., carbohydrate antigen 19-9, carcinoembryonic antigen) should also be evaluated for early detection of cholangiocarcinoma⁴.

Conclusions

Periodic health examinations, especially those aimed at assessing serum liver enzyme levels, should be conducted for 1,2-DCP exposed workers not only during exposure but also after termination of exposure. In examinations, even small increases in γ -GTP levels should be considered a sign of early development of cholangiocarcinoma.

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