

分担研究報告書

9. ジクロロメタン取扱事業所における作業環境測定結果について

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研究要旨

ジクロロメタン取扱事業所9か所の作業環境測定を実施した。その結果、適切とされている第1管理区分は6事業所、なお改善の余地があるとされている第2管理区分は1事業所、適切でない第3管理区分は2事業所であった。A測定が区分Ⅰのものは7事業所、区分Ⅱのものは2事業所、B測定が区分Ⅰのものは7事業所、区分Ⅲのものは2事業所であった。このうちB測定区分Ⅲのものは第3管理区分とされている。局所排気装置あるいは密閉装置を用いていない事業所は5事業場でそのうち2事業場はオフセット印刷作業に伴い行われるローラーの払拭を行っていた。作業環境測定時の使用量、作業頻度、単位作業場の広さ等の影響はあるものの、管理区分を適切に維持するには局所排気装置や密閉等の設備が必要であり、またそれらの適切な管理維持が重要であると考えられた。

A. 研究目的

ジクロロメタンは不燃性、高い溶解性等の優れた産業特性を持ち、脱脂洗浄等の用途で普遍的に用いられている。ジクロロメタンは有機溶剤中毒防止規則が定める第2種有機溶剤(管理濃度 50ppm)であるが、これまでその健康影響等については急性中毒事例を除いて考察される機会は少なかった。しかし、2012年に熊谷らがジクロロメタンに起因する職業性胆管がんの報告を行って以来、産業現場におけるジクロロメタンジクロロメタンの取り扱い状況やその作業環境管理の実態に関心が集まっている。今回、我々は平成26年度厚生労働科学研究「印刷労働者にみられる胆管癌発症の疫学的解明と原因追究」の分担研究においてジクロロメタン取扱事業所で行った作業環境測定に関する結果のとりまとめを行ったので、その報告を行う。

B. 研究方法

平成24年6月から平成25年10月にかけて、関西労働衛生技術センターが作業環境測定を実施した関西地方のジクロロメタン取扱事業所9か所(内訳: 製薬1、印刷3、機械・製造5、単位

作業場の広さ:6-217 m²、ジクロロメタンの製造または取扱量: 4-500kg/月)を対象とした。試料の採取方法はテドラーバッグ、捕集器具にMSAポータブルポンプを用いた直接捕集法を用いて行い、条件は吸引流量 0.5L/min、捕集時間 10分間、捕集量 5L とした。単位作業場所あたりのA測定の測定点の数は5-7、サンプリング時間は60分、B測定のサンプリング時間は10分とした。分析は日立263-50によるガスクロマトグラフィー法を用いた。

C. 研究結果

表及び図に示すように9事業所のうち第1管理区分(適切)は6事業所、第2管理区分(なお改善の余地)は1事業所、第3管理区分(適切でない)は2事業所であった。図で示すようにA測定が区分Ⅰのものは7事業所、区分Ⅱのものは2事業所、B測定が区分Ⅰのものは7事業所、区分Ⅲのものは2事業所であった。このうちB測定区分Ⅲのものは第3管理区分とされている。A測定あるいはB測定の区分がⅡあるいはⅢとなるオッズ比は局所排気装置なしの群で3倍、管理区分が2あるいは3となるオッズ比は局所

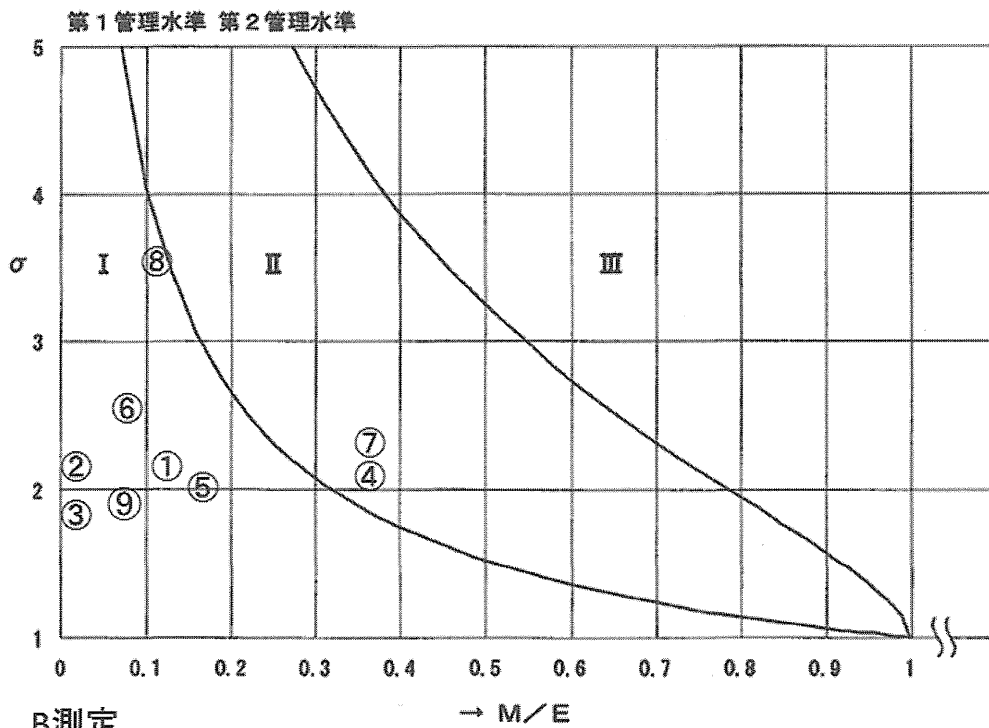
排気装置なしの群で2倍となった。

表 関西地方のジクロロメタン取扱事業所9か所における作業環境測定結果

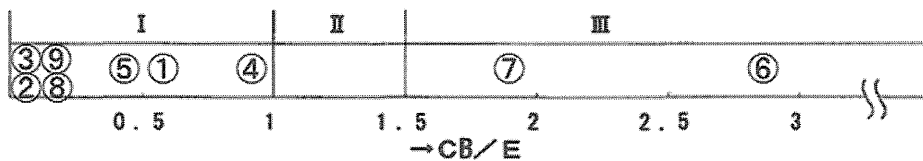
事業所	設備	管理区分	A測定				B測定		
			幾何平均値 M	幾何標準偏差 σ	第1評価値 E_{A1}	第2評価値 E_{A2}	区分	C_B	区分
①	局所排気装置稼働 あるいは密閉型	1	5.9	2.12	20.3	7.9	I	27.0	I
②		1	1.1	2.09	3.8	1.6	I	1.0	I
③		1	0.5	1.95	1.49	0.63	I	1.5	I
④	局所排気装置なし	2	18.4	2.06	60.5	23.9	II	48.0	I
⑤		1	9.2	1.99	28.5	11.7	I	24.0	I
⑥		3	3.9	2.40	16.3	5.7	I	146.0	III
⑦		3	18.6	2.08	61.7	24.3	II	94.0	III
⑧		1	0.081	2.00	0.251	0.103	I	0.12	I
⑨		1	5.9	3.55	47.0	13.1	I	4.0	I

図 関西地方のジクロロメタン取扱事業所9か所における作業環境測定結果

A測定



B測定



D. 考察

局所排気装置あるいは密閉装置を用いない場

合、ジクロロメタンの気中濃度が上昇し作業環境管理の適切性に影響を与えることが示唆され

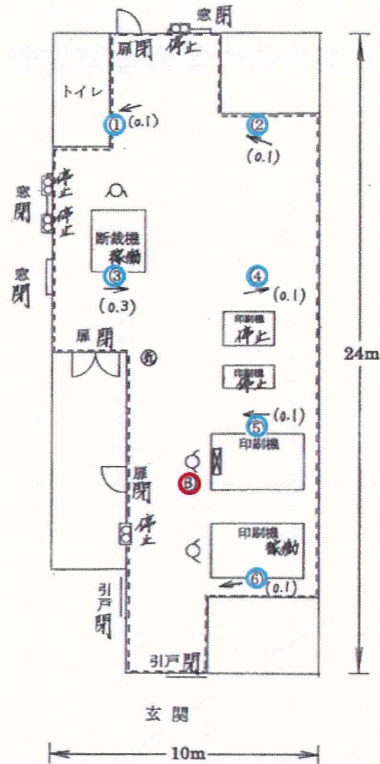
た。

局所排気装置あるいは密閉装置を用いていない事業所⑤-⑨を個別に考察したところ、事業所⑤、⑦ではオフセット印刷作業に伴い行われ

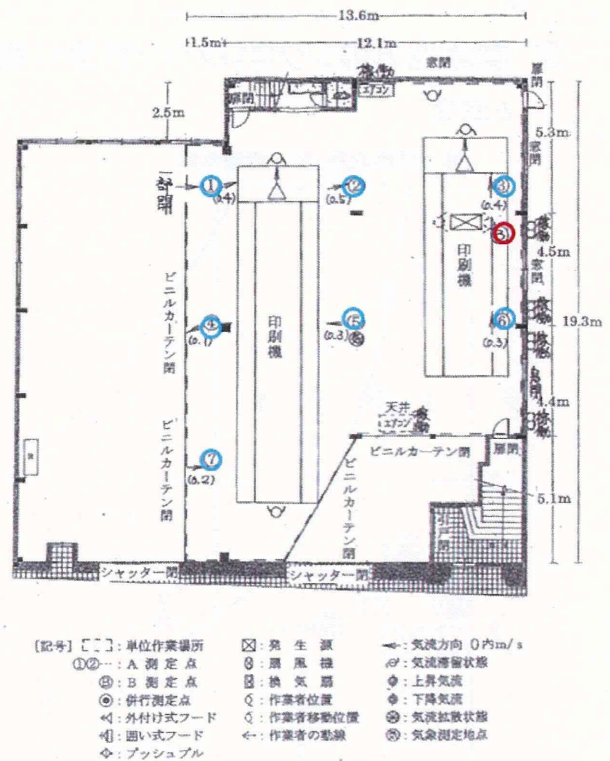
るローラーの払拭を行っていた。

事業所⑤では作業頻度が少なく、事業所⑦では作業が断続的であったため測定結果に差が生じたと考えられた。

事業所⑤ 管理区分1
A測定 区分Ⅰ B測定 区分Ⅰ
作業場面積 172m²、取扱量10kg/月



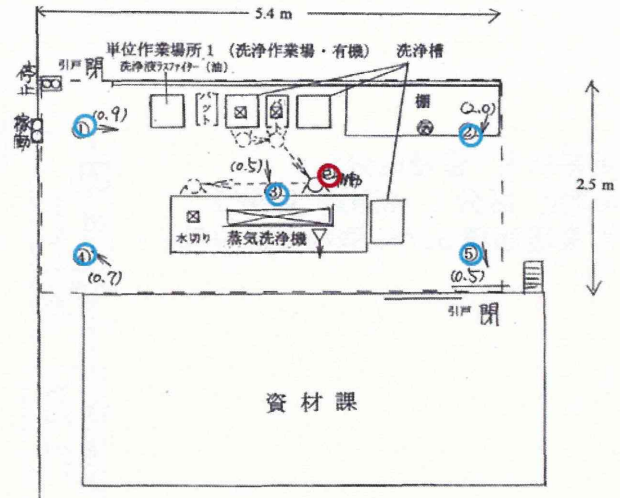
事業所⑦ 管理区分3
A測定 区分Ⅱ B測定 区分Ⅲ
作業場面積 217m²、取扱量8kg/月



事業所⑥では機械部品の洗浄作業を行っていたが、洗浄槽から蒸気洗浄槽へ洗浄物を移動する作業工程、また使用量が多く(500kg/月)、

作業場が狭小(14 m²)であることが濃度上昇に関与したと考えられた。

事業所⑥ 管理区分3
 A測定 区分Ⅰ B測定 区分Ⅲ
 作業場面積 14m²、取扱量500kg/月



- [記号] □□: 単位作業場所
 ①②...: A 測定点
 ③: B 測定点
 ④⑤: 併行測定点
 ◀: 外付け式フード
 ◀◀: 囲い式フード
 ◆: プッシュプル
 □: 発生源
 ⊗: 扇風機
 ⊕: 換気扇
 ○: 作業者位置
 ○: 作業者移動位置
 ←: 作業者の動線
 ←: 気流方向 〇内m/s
 ⊙: 気流滞留状態
 ⊕: 上昇気流
 ⊖: 下降気流
 ⊗: 気流拡散状態
 ⊙: 気象測定地点

事業所⑧では銅線被膜の剥離作業を行っていたが使用量の少なかつた(2.5kg/月)ことから濃度

上昇が抑制されたと考えられた。

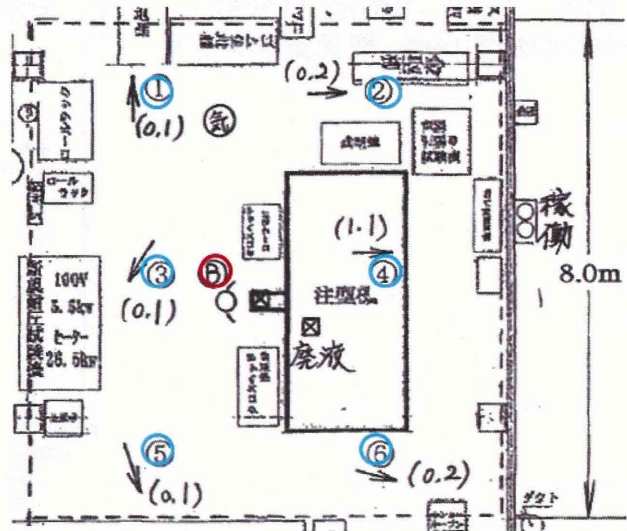
事業所⑧ 管理区分1
 A測定 区分Ⅰ B測定 区分Ⅰ
 作業場面積 187m²、取扱量2.5kg/月



事業所⑨ではウレタン注型器のノズル洗浄作業を行っていたが使用量や作業頻度が少なく(6kg/月で測定時の作業は1回のみ)、ノズルと廃

液容器が接続されていたことから濃度上昇が抑制されたと考えられた。

事業所⑨ 管理区分1
 A測定 区分I B測定 区分I
 作業場面積 62㎡、取扱量6kg/月



事業所④ 管理区分2
 A測定 区分II B測定 区分I
 作業場面積 155㎡、取扱量200kg/月

事業所④では局所排気装置が設置されていたが、A測定の①が高値、B測定がやや高値となった。

これはウレタン注入機のノズル洗浄作業位置付近に設置されている扇風機や天井エアコンの風の影響が考えられた。



不適切な空調例



(参考写真、事業場④とは関係ありません)

- (記号)
- ①② A測定点
 - ③ B測定点
 - ④ 併行測定点
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 - ⑥ 併行測定点
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E. 結論

作業環境測定時の使用量、作業頻度、単位作業場の広さ等の影響はあるものの、管理区分を適切に維持するには局所排気装置や密閉等の設備が必要であると考えられた。局所排気装置が設置されている場合でも不適切な設置例や、送風機・気流の影響を受けている場合もありB測定において高値となる例も見られた。

平成24年7月1日からは局所排気装置またはプッシュプル型換気装置以外の多様な発散防止抑制装置の導入の特例が認められているが、局所排気装置を含め作業場の適切な管理維持を行うには衛生管理者・産業医の定期的な巡視を含め労働安全衛生法の順守が必要である。

F. 健康危険情報

特になし

G. 研究発表

1. 論文発表 なし
2. 学会発表 なし

H. 知的財産権の出願・登録状況(予定を含む)

1. 特許取得 なし
2. 実用新案登録 なし
3. その他 なし

III. 研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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<u>Kubo S</u> , Takemura S, Sakata C, Urata Y, Nishioaka T, Nozawa A, Kinoshita M, Hamano G, Nakamura Y, <u>Endo G</u>	Changes in laboratory test results and diagnostic imaging presentation before the detection of occupational cholangiocarcinoma	J Occup Health		(in press)	2014
<u>Kubo S</u> , Nakanuma Y, Takemura S, Sakata C, Urata Y, Nozawa A, Nishioaka T, Kinoshita M, Hamano G, Terajima H, Tanachiyama G, Matsumura Y, Yamada T, Tanaka H, Nakamori S, Arimoto A, Kawada N, Fujikawa M, Fujishima H, Sugawara Y, Tanaka S, Toyokawa H, Kuwae Y, Ohswa M, Uehara S, Sato KK, Hayashi T, <u>Endo G</u> .	Case series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan.	J Hepatobiliary Pancreat Sci.		[Epub ahead of print]	2014
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Case series of 17 patients with cholangiocarcinoma among young adult workers of a printing company in Japan

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Chikaharu Sakata · Yorihisa Urata · Akinori Nozawa ·
Takayoshi Nishioka · Masahiko Kinoshita ·
Genya Hamano · Hiroaki Terajima · Gorou Tachiyama ·
Yuji Matsumura · Terumasa Yamada · Hiromu Tanaka ·
Shoji Nakamori · Akira Arimoto · Norifumi Kawada ·
Masahiro Fujikawa · Hiromitsu Fujishima ·
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Abstract

Background An outbreak of cholangiocarcinoma occurred among workers in the offset color proof-printing department at a printing company in Japan. The aim of this study was to clarify the characteristics of the patients with cholangiocarcinoma.

Methods This was a retrospective study conducted in 13 Japanese hospitals between 1996 to 2013. The clinicopathological findings of cholangiocarcinoma developed in 17 of 111 former or current workers in the department were investigated. Most workers were relatively young.

Results The cholangiocarcinoma was diagnosed at 25–45 years old. They were exposed to chemicals, including dichloromethane and 1,2-dichloropropane. The serum γ -glutamyl transpeptidase activity was elevated in all patients. Dilated intrahepatic bile ducts without tumor-induced obstruction were observed in five patients. The cholangiocarcinomas arose from the large bile ducts. The precancerous or early cancerous lesions, such as biliary intraepithelial neoplasia and intraductal papillary neoplasm of the bile ducts, as well as non-specific bile duct injuries, such as fibrosis, were observed in various sites of the bile ducts in all eight patients for whom operative specimens were available.

Conclusions The present results showed that cholangiocarcinomas occurred at a high incidence in relatively young workers of a printing company, who were exposed to chemicals including chlorinated organic solvents.

Keywords Biliary intraepithelial neoplasia · Cholangiocarcinoma · Intraductal papillary neoplasm of the bile duct · Organic solvent · Printing company

Introduction

Cholangiocarcinoma, a relatively rare cancer, arises from the biliary epithelium of the liver (intrahepatic cholangiocarcinoma) or in the extrahepatic bile ducts (extrahepatic cholangiocarcinoma) [1]. The peak age of cholangiocarcinoma diagnosis is within the seventh decade of life, and the disease occurs slightly more frequently in men [2–6]. Cholangiocarcinoma incidence rates vary markedly throughout the world, which presumably reflects differences in local risk factors and genetics. Although the incidence in the United States is 1–2 cases per 100,000 individuals, the highest rates of cholangiocarcinoma occur in Northeast Thailand (96 per 100,000 men) [3, 7, 8].

Recently, Kumagai et al. reported 11 cases of cholangiocarcinoma developing in former or current workers in the offset color proof-printing department at a printing company in Osaka, Japan [9]. However, they did not survey all of the patients with cholangiocarcinoma in the company or investigate any clinicopathological characteristics of the patients.

In this study, we describe the clinical findings, laboratory test results, diagnostic imaging results, pathological findings, treatments, and prognosis of all patients with cholangiocarcinoma who were former or current workers of the printing company because this information is necessary to publicize to detect cholangiocarcinoma as a possible occupational disease and to prevent subsequent occurrence. We also discuss the possible carcinogenic progression of cholangiocarcinoma.

Subjects and methods

The subjects were 17 men with cholangiocarcinoma who were former or current workers at an offset color proof-printing department at a printing company in Osaka, Japan (Table 1). Cholangiocarcinoma was diagnosed between November 1996 and November 2012. Various types of chemicals, including chlorinated organic solvents such as 1,1,1-trichloroethane, dichloromethane, and 1,2-dichloropropane were used to clean ink residues in this department (Table S1). The chemicals used in this department have been changed several times. 1,1,1-Trichloroethane was used until December 1992, dichloromethane was used until March 1996, and 1,2-dichloropropane was used until October 2006. Various types of inks have also been used. This department was estimated to have 111 former or current workers (88 men and 23 women) between 1981 and 2012. Most workers were relatively young; a few workers were more than 50 years old and they were not exposed to high concentration of chlorinated organic solvent. Ten of the 111 workers could not be followed up after the resignation from the company. The clinical findings, laboratory test results, diagnostic imaging results, pathological findings, treatments and prognosis of the 17 patients were investigated. The 17 patients were treated at 13 hospitals. The information concerning clinical findings, including history of alcohol intake and smoking, were obtained from the medical records of each hospital and/or interviews with the patients. The laboratory test results were obtained from the medical records. For diagnostic imaging, computed tomography (CT) and ultrasonography were performed in all patients and magnetic resonance imaging (MRI), including magnetic resonance cholangiopancreatography (MRCP), was performed in 15 patients. Direct cholangiograms were obtained by endoscopic retrograde cholangiopancreatography (ERCP) in nine patients and during percutaneous transhepatic biliary drainage (PTBD) in two patients. The films of diagnostic images were available in 13 patients, and the imaging reports in the medical records were reviewed in the remaining four patients because the films had been retired. For 16 of the 17 patients, the pathological diagnosis was made by pathologists in the individual hospitals using the

Table 1 Clinical findings in patients with cholangiocarcinoma

Patient no.	Clinical findings				Laboratory tests			Diagnostic imaging	Diagnosis of cholangiocarcinoma		Treatments and prognosis after diagnosis	
	Age/sex	Symptom or health examination	Alcohol abuse	Smoking	γ -GTP (IU/l)	CEA (ng/ml)	CA19-9 (U/ml)	Methods for clinical diagnosis	Location and type of cholangiocarcinoma	Stage by TNM classification ¹⁾	Treatments	Prognosis
1	34/M	Epigastralgia, back pain	No	Yes	830	35.9	15200	Cholangiography during PTBD	ICC, mass-forming	IVA	Extended rt. hepatectomy, resection of extrahepatic bile duct, chemotherapy	7 years, 3 months, Dead
2	34/M	Rt. hypochondralgia	No	Yes	785	38.0	114	US, CT	ICC, mass-forming	IVA	Rt. trisectionectomy	1 year, 2 months, Dead
3	29/M	Jaundice, appetite loss	No	Yes	264	2.0	505	MRI	ECC, papillary	1B	Resection of extrahepatic bile duct	1 year, 7 months, Dead
4	25/M	Liver dysfunction	Yes	Yes	1729	8832.0	30.5	US, CT	ICC, mass-forming	IVB	Chemotherapy	1 year, 7 months, Dead
5	35/M	Liver dysfunction	No	Yes	2457	1.6	119.2	US, CT	ECC, papillary	IVA	Rt. hepatectomy, resection of extrahepatic bile duct ^c	2 years, 3 months, Dead
6	45/M	Rt. hypochondralgia, jaundice	No	Yes	1570	199.4	216394	US, CT	ECC, nodular	IVB	Conservative treatment	1 year, Dead
7	40/M	Liver dysfunction	No	Yes	1049	1.0	34	US, CT	ICC, mass-forming ECC, papillary carcinoma of Papilla of Vater	IIA ^b	Rt. hepatectomy, pancreaticoduodenectomy	6 years, 5 months, Alive
8	38/M	Liver tumor	No	No	208	2.9	2288	US, CT	ICC, mass-forming	IVA	Segmentectomy 8, chemotherapy	5 years, 8 months, Alive
9	39/M	Epigastralgia, weight loss	No	Yes	1983	28.6	4	US, MRI	ICC, mass-forming	IVB	Chemotherapy	9 months, Dead
10	40/M	Liver dysfunction ^a	No	Yes	1037	5.5	446	MRI	ICC, intraductal growth	IVA	Lt. hepatectomy ^c , radiation, chemotherapy	3 years, 2 months, Dead
11	31/M	Liver dysfunction	No	Yes	1196	5.1	1084	US, CT	ECC, papillary	IVA	Extended rt. hepatectomy ^c , chemotherapy	3 years, 3 months, Alive
12	39/M	Liver dysfunction	No	No	486	5.4	20.6	ERCP	ICC, intraductal growth ECC, papillary	IVA ^b	Lt. hepatectomy, radiation, chemotherapy	3 years, Alive
13	44/M	Liver dysfunction, liver tumor	Yes	Yes	1890	17.7	7749	US, CT	ICC, mass-forming	IVB	Chemotherapy	9 months, Dead
14	37/M	Liver dysfunction	Yes	Yes	451	9.6	50.5	MRI	ECC, nodular	IVB	Chemotherapy	1 year, 3 months, Alive
15	39/M	Liver dysfunction	No	Yes	347	8.2	105	ERCP	ICC, intraductal growth	IVA	Lt. hepatectomy, segmentectomy 7, chemotherapy	1 year, 1 month, Alive
16	31/M	Liver dysfunction	No	No	75	2.1	501	US, CT	ICC, mass-forming	IVA	Rt. hepatectomy, resection of extrahepatic bile duct ^c , chemotherapy	1 year, Alive
17	34/M	Liver dysfunction	No	No	205	5	54	US, CT	ICC, intraductal growth	IVA	Extended lt. hepatectomy	11 months, Alive

γ -GTP γ -glutamyl transpeptidase, CA19-9 carbohydrate antigen 19-9, CEA carcinoembryonic antigen, CT computed tomography, ECC extrahepatic cholangiocarcinoma, ERCP endoscopic retrograde cholangiopancreatography, ICC intrahepatic cholangiocarcinoma, MRI magnetic resonance imaging, PTBD percutaneous transhepatic biliary drainage, US ultrasonography

^a Liver dysfunction was detected during treatment for paranasal sinusitis

^b Most advanced stage

^c Non-curative resection

The stage of the tumors in patients 7 and 12 could not be classified because the patients had intra and extrahepatic cholangiocarcinomas

operative specimens in 12 patients and the biopsy specimens in four patients. The pathology examination could not be performed in another patient (patient no. 6); the diagnosis of cholangiocarcinoma was made by diagnostic imaging in this patient. The clinical findings, laboratory test results, diagnostic imaging results, and clinical course were re-evaluated by the medical staff (S.K., S.T., C.S., Y.U., A.N., T.N., M.K., G.H.) of Osaka City University Hospital. The operative specimens were available from 8 of the 12 patients treated surgically and were examined further by Y.N.

The pathological findings were recorded and described according to the World Health Organization's classifications for intrahepatic and extrahepatic cholangiocarcinoma [1]. Intrahepatic cholangiocarcinoma was grossly classified as mass-forming, periductal infiltrating, or intraductal growth (IG). Extrahepatic cholangiocarcinoma was grossly classified as papillary, nodular, or diffuse infiltrating. Preneoplastic or early preinvasive neoplastic lesions of the biliary tree were classified as flat dysplastic epithelium (biliary intraepithelial neoplasia; BilIN) or grossly visible papillary types (intraductal papillary neoplasm of the bile duct; IPNB). BilIN lesions were histologically classified according to their cellular and structural features as BilIN-1 (mild atypia), BilIN-2 (moderate atypia), or BilIN-3 (severe atypia corresponding to *in situ* carcinoma). In this study, BilIN-2 and BilIN-3 lesions were mainly surveyed because whether BilIN-1 lesions contain some reactive hyperplastic changes remains controversial. The classification of BilIN is described in Table 2. Cases of invasive carcinoma associated with IPNB (invasive IPNB) were classified as the IG

type of intrahepatic cholangiocarcinoma or papillary type of extrahepatic cholangiocarcinoma [1, 10–12]. Other pathological terms used in this study were characterized or defined as follows. "Chronic bile duct injury" was used as a collective term of duct injuries such as epithelial damages, fibrosis of duct wall and periductal tissue, and chronic inflammatory cell infiltration in various combinations. "Proliferative changes of bile ducts" were used for the bile ducts with non-neoplastic biliary epithelial proliferation. "Bile duct sclerosis" indicates fibrous thickening of duct wall with or without additional periductal fibrosis.

This study was approved by the ethics committee of Osaka City University, and all of the subjects or their legally authorized representatives (for deceased patients) provided written informed consent. The multicenter occupational cholangiocarcinoma study group consisted of investigators in 13 hospitals (including four university hospitals) and two universities.

Results

Clinical findings

The age of the patients at cholangiocarcinoma diagnosis ranged from 25 to 45 years old (mean, 36 years old; Table 1). They started to work at the printing company when they were 18 to 28 years old. The cholangiocarcinoma was diagnosed in 10 current and seven former workers. The period from the start of employment until diagnosis of cholangiocarcinoma or resignation from the company, which was considered the period of exposure to chemicals prior to the diagnosis of cholangiocarcinoma, ranged from 6 years, 1 month to 19 years, 9 months (median: 11 years, 4 months; Fig. S1). The longest period between the end of the exposure and the detection of cholangiocarcinoma was 9 years, 7 months. Of the 17 patients, all were exposed to 1,2-dichloropropane, 11 were exposed to dichloromethane, and eight were exposed to 1,1,1-trichloroethane. The period of exposure to chlorinated organic solvent ranged from 6 years, 1 month to 16 years, 1 month (median: 9 years, 7 months).

Of the seven patients in whom carcinomas were detected after resignation from the company, two were employed at a different printing company that did not use 1,1,1-trichloroethane, dichloromethane, or 1,2-dichloropropane, and five were employed in jobs without usage of chemicals.

Of the 17 patients, five patients suffered from abdominal pain, jaundice, and/or appetite loss. Abnormal liver function test results or liver tumors were detected in 11 patients during health examinations. In another patient (no. 10), liver dysfunction was detected during treatment for paranasal sinusitis. Four patients were non-drinkers, three patients were habitual alcohol consumers (≥ 80 g of ethanol daily) [13], and 13 patients were smokers.

Table 2 Pathological findings in the operative specimens

Patient no.	Chronic bile duct injury	Proliferative changes in bile ducts	BilIN-2/3	IPNB/invasive IPNB	Main tumor metastasis	Lymph node
2	+	+	+	+	MF, poorly	-
8	+	+	+	ND	MF, mode	+
10	+	+	+	+	IG, well	+
11	+	+	+	+	pap, well	-
12	+	+	+	+	IG, pap, well	-
15	+	+	+	+	IG, well	-
16	+	+	+	+	MF, mode	+
17	+	+	+	+	IG, well	-

ND not determined because of small noncancerous hepatic tissue

Chronic bile duct injury was a collective lesion of various injuries such as epithelial damages, fibrosis of duct wall and periductal tissue, and chronic inflammatory cell infiltration. Proliferative changes were characterized by non-neoplastic biliary epithelial proliferation. BilIN, biliary intraepithelial neoplasia; BilIN-1 lesions presented with mild atypical cellular and nuclear features such as nuclear membrane irregularities or nuclear enlargements with only minimal disturbances to cellular polarity. BilIN-2 had evident aberrant cellular and nuclear features not sufficient to suggest overt carcinoma and focal disturbances in cellular polarity. BilIN-3 presented with diffuse disturbances in cellular polarity with or without distinct atypical cellular and nuclear features that corresponded to carcinoma *in situ*. In this study, BilIN-2/3 lesions were surveyed. IPNB, intraductal neoplasm of the bile; MF, mass-forming type intrahepatic cholangiocarcinoma; IG, intraductal growth type intrahepatic cholangiocarcinoma; pap, papillary type extrahepatic cholangiocarcinoma; poorly, poorly differentiated adenocarcinoma; mode, moderately differentiated adenocarcinoma; well, well-differentiated adenocarcinoma

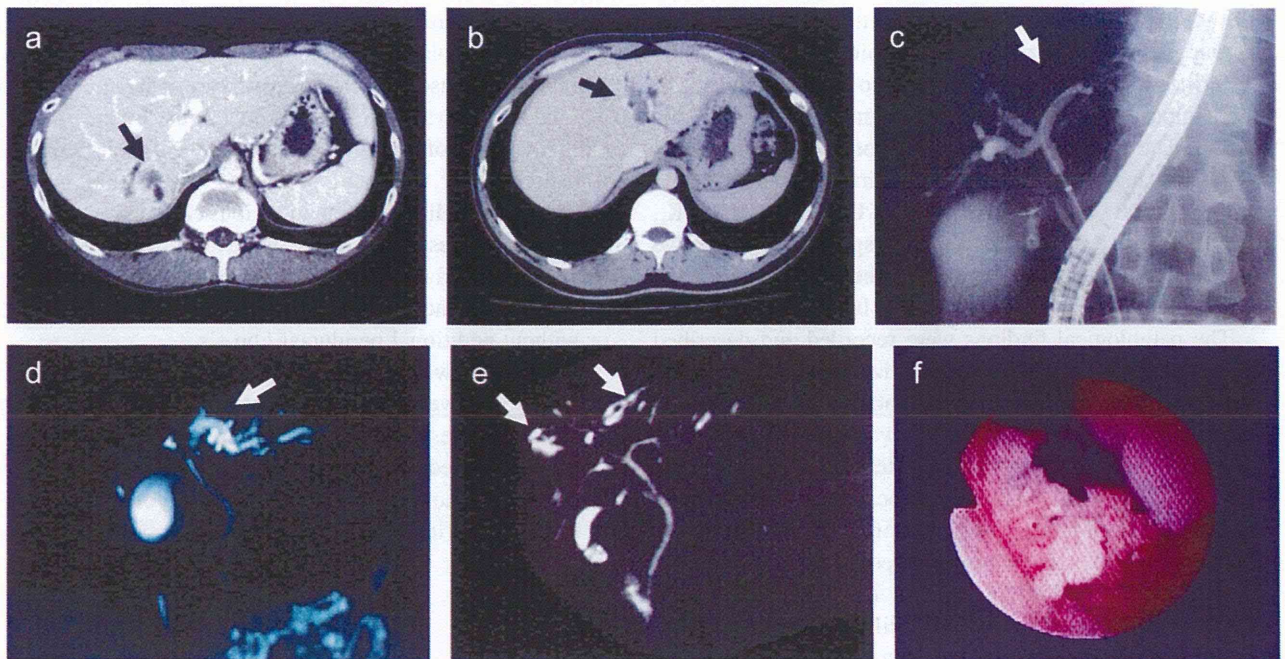


Fig. 1 Diagnostic imaging of patients with cholangiocarcinomas

(a) Intrahepatic cholangiocarcinoma of the mass-forming type (arrow), patient 16; (b) a dilated intrahepatic bile duct with a papillary tumor (intraductal growth type, arrow), patient 12; (c) stenosis of the intrahepatic bile duct due to cholangiocarcinoma (arrow), patient 15; (d) dilated intrahepatic bile ducts due to cholangiocarcinoma (arrow), patient 10; (e) dilated intrahepatic bile ducts without tumor-induced obstruction (arrow), patient 15; (f) intraoperative cholangiofiberscopy exhibiting lesions protruding into the bile duct, patient 15

Laboratory test results

At the time of cholangiocarcinoma diagnosis, the serum concentrations of total bilirubin were elevated in eight patients. Serum activities of aspartate and alanine aminotransferase were elevated in 13 patients and 14 patients, respectively. The serum γ -glutamyl transpeptidase (γ -GTP) activity was elevated in all of the patients (Table 1). In the five patients with available laboratory test results from several years prior to the diagnosis of cholangiocarcinoma, the serum γ -GTP activity was consistently high. The serum concentrations of carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) were elevated in 11 and 13 patients, respectively. The tests for serum hepatitis B surface antigen and hepatitis C virus antibodies were negative in all of the patients. A test for the hepatitis B core antibody was positive in one of the 11 patients examined.

Diagnostic imaging

Clinical diagnosis by imaging was made first by ultrasonography, CT, MRI, ERCP, and/or cholangiograms during PTBD (Table 1). Space-occupying lesions were demonstrated in nine patients (no. 1, 2, 4, 6–9, 13, and 16) using CT, MRI, and/or ultrasonography (Fig. 1a). Bile ducts with papillary, villous, or protruding tumors were observed in five

patients (no. 3, 5, 11, 12, and 17) on ultrasonography, CT, and/or ERCP (Fig. 1b). Stenosis or obstructions of the bile duct were observed in five patients (no. 1, 7, 10, 11, and 15; Fig. 1c) as observed on MRCP and/or direct cholangiograms. By contrast, the dilatation of peripheral bile ducts due to tumor-induced bile duct obstruction was observed in 11 patients (no. 1, 2, 4–7, and 9–14) in ultrasonography, CT and/or MRI (Fig. 1d). Dilated intrahepatic bile ducts without tumor-induced obstructions were observed in five patients (no. 8, 12, and 15–17) on ultrasonography, CT, and/or MRI (Fig. 1e). In these five patients, findings that were noted on cholangiography appeared to correspond to those of primary sclerosing cholangitis (PSC), including multiple intrahepatic bile duct strictures with or without fusiform dilatation [14]. Intraoperative cholangiofiberscopy, which was performed on three patients (no. 15–17), revealed epithelial irregularities of the bile ducts, IPNB, and papillary lesions protruding into the dilated bile ducts without tumor-induced obstruction (Fig. 1f).

Diagnosis of cholangiocarcinoma

Intrahepatic cholangiocarcinoma was identified in 10 patients, extrahepatic cholangiocarcinoma was identified in five patients, and both intrahepatic and extrahepatic cholangiocarcinomas were identified in two patients during

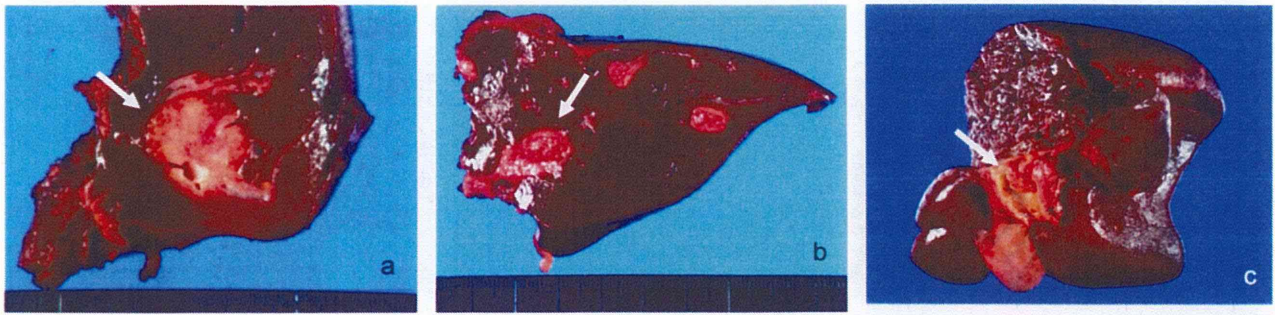
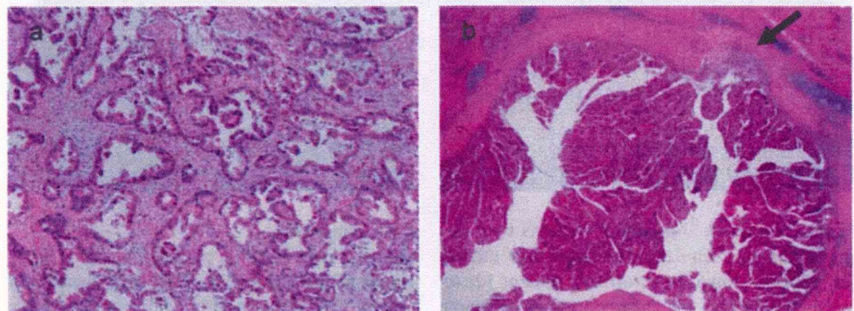


Fig. 2 Operative specimens

(a) intrahepatic cholangiocarcinoma of the mass-forming type (arrow), patient 16; (b) intrahepatic cholangiocarcinoma of the intraductal growth type (arrow), patient 15; (c) extrahepatic cholangiocarcinoma (hilar cholangiocarcinoma) of the papillary type (arrow), patient 11

Fig. 3 Histological features of cholangiocarcinomas

(a) Intrahepatic cholangiocarcinoma of the mass-forming type with tubular adenocarcinoma, patient 8, hematoxylin and eosin (H-E) staining, 400 × magnification; (b) Intrahepatic cholangiocarcinoma of the intraductal growth type with focal invasion (arrow) (corresponding to intraductal papillary neoplasm of the bile duct with an associated invasive carcinoma), patient 12, H-E staining, 150 × magnification



diagnostic imaging and/or surgery (Table 1). Among the 12 patients with intrahepatic cholangiocarcinoma, the tumors were classified as mass-forming type in eight patients (Fig. 2a) and as IG type in four patients (Fig. 2b). Among the seven patients with extrahepatic cholangiocarcinoma, the tumors were classified as papillary-type cholangiocarcinoma (Fig. 2c) in five patients and as nodular-type in two patients. Surgically resected specimens or biopsy specimens revealed variably differentiated adenocarcinomas compatible with cholangiocarcinoma in all 16 patients in whom pathological examination was performed. Comprehensive imaging studies and gross analyses of these patients demonstrated that the main and most invasive cholangiocarcinoma lesions were located in the common hepatic duct, the left or right hepatic duct, or the first to third branches of the intrahepatic bile duct (also known as the large bile duct [15]).

Histopathological findings

The operative specimens were available from eight patients in this study (Table 2). Among the eight patients, three patients with mass-forming type of intrahepatic

cholangiocarcinoma showed well-, moderate or poor adenocarcinoma (no. 2, 8, 16; Fig. 3a), and five patients with IG-type of intrahepatic cholangiocarcinoma and/or papillary-type extrahepatic cholangiocarcinoma exhibited well-differentiated papillary carcinoma (invasive IPNB) (no. 10–12, 15, 17; Fig. 3b). In the latter group, the invasive portions of the tumors were mucinous or tubular adenocarcinoma. Additionally, IPNB lesions without invasion were grossly and histologically detected in the various sites of the resected specimens from three patients (no. 2, 15, 16). Extensive intraductal spread of non-invasive neoplastic biliary epithelial cells, focally intraductal papillary pattern, was observed in three patients (no. 2, 15, 16). BilIN-1 and also BilIN-2/3 lesions were detected in various sites of the large intrahepatic bile ducts and/or hilar bile ducts and the peribiliary glands in all eight patients (Figs. 4a–c). Peribiliary glands, when they were identified in the specimens, showed hyperplastic changes and also atypical and preneoplastic lesions, corresponding to BilIN-2/3 lesions (Fig. 4d,e). In all eight patients, sclerosis of the bile duct with variable inflammatory cell proliferation, biliary epithelial injuries/focal bile duct loss, and biliary epithelial hyperplasia were also observed in various sites of

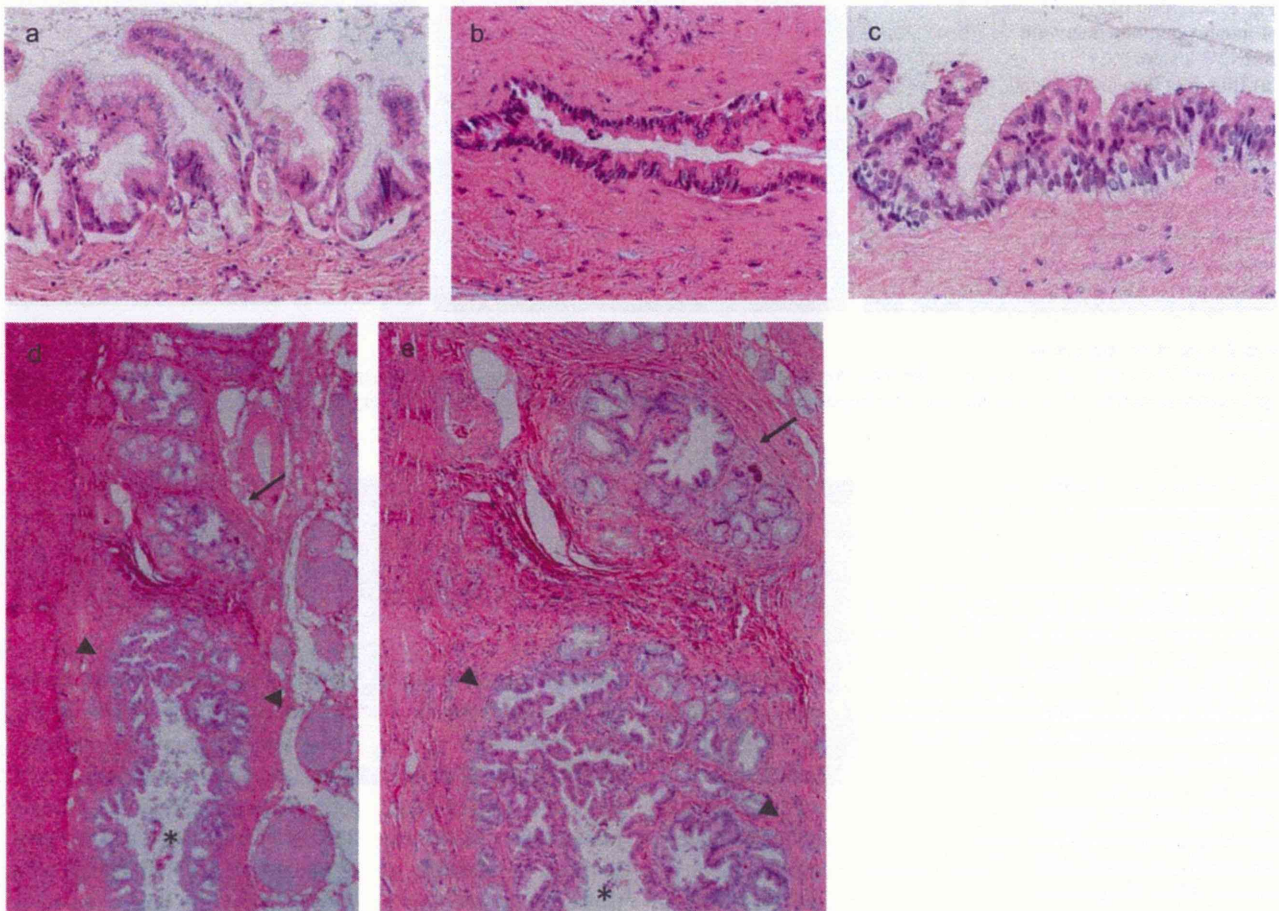


Fig. 4 Histological features of biliary intraepithelial neoplasia (BilIN) of bile ducts. (a) BilIN-1, patient 2. H-E staining, 250 × magnification. (b) BilIN-2, patient 12, H-E staining, 300 × magnification. (c) BilIN-3, patient 12, H-E staining, 300 × magnification. (d) Peribiliary glands of intrahepatic large bile duct (*) showing hyperplastic changes and foci of mild atypia, corresponding to BilIN-2 lesion, in the bile duct wall (arrow heads) and in the surrounding tissue (arrow), patient 10, H-E staining, 150 × magnification. (e) Higher magnification of D. H-E staining, 250 × magnification

the bile ducts in the noncancerous hepatic tissues (Fig. 5a,b). As for the pathology of the non-neoplastic, background liver, non-specific reactive changes or cholestatic changes secondary to obstruction or stenosis of bile ducts affected by cholangiocarcinoma were observed. Lymph node metastasis in the hepatoduodenal ligament and/or the along the common hepatic artery was found in three patients.

In summary, the precancerous or early cancerous lesions, such as BilIN and IPNB, as well as non-specific 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 patients. Invasive carcinoma was observed in the operative specimens.

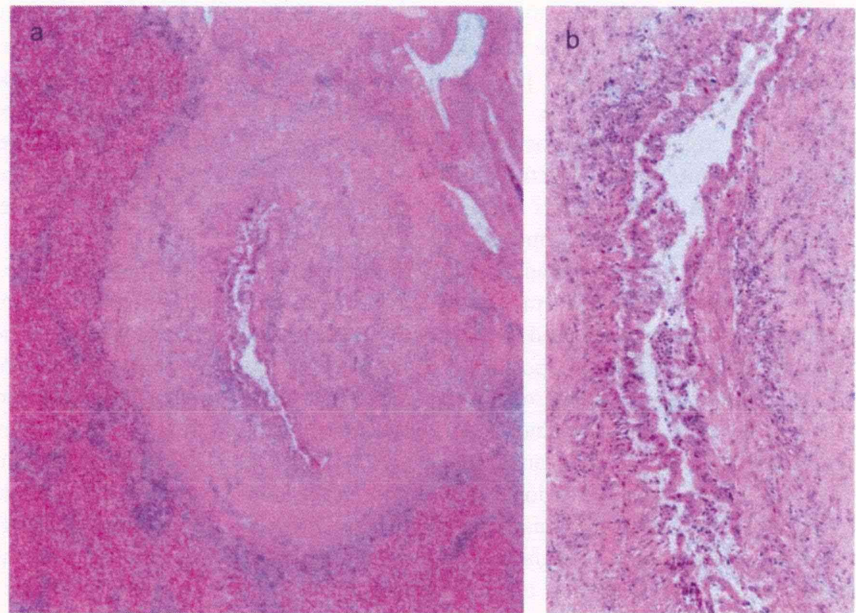
No cirrhotic changes or other hepatobiliary diseases were detected in the noncancerous hepatic tissues of the eight patients. Thus, the laboratory test results, diagnostic imaging results, and/or pathological findings indicated

that the 17 patients did not have any known risk factors for cholangiocarcinoma, such as PSC, hepatolithiasis, pancreaticobiliary maljunction, or liver fluke infection (*Clonorchis sinensis* and *Opisthorchis viverrini*) [2–4, 16–19].

Treatment and prognosis

Surgical resection was performed in 12 patients (Table 1). Curative resection could not be performed for 4 of these 12 patients because of the detection of cancer cells in the resected stumps of the bile ducts. In 10 of the 12 patients, dissection or sampling of the lymph nodes was performed. Four (no. 1, 8, 10, and 16) of the 10 patients exhibited metastases to the lymph nodes around the common bile duct or the common hepatic artery and/or peripancreatic lesions. Adjuvant chemotherapy with fluorouracil, gemcitabine, and/or S-1 (tegafur/gimeracil/oteracil potassium) was

Fig. 5 Sclerotic bile duct lesion of intrahepatic bile duct with BilIN2/3 lesions
 (a) Bile duct prominent ductal sclerosis with stenotic lumen. Patient 8, H-E staining, 150 × magnification. (b) Higher magnification of (a) Stenotic lumen covered by BilIN2/3 lesions. Beneath the epithelial lesion, mesenchymal reaction with inflammatory cells is seen. H-E staining, 150 × magnification; H staining, 300 × magnification; H



administered to eight patients after surgical treatment. In two of these eight patients, radiation was administered to the bile duct stumps. In the remaining 5 of the 17 patients, chemotherapy or conservative treatment (stenting alone in one patient) was administered because of the advanced stage of the disease, as indicated by metastases to the lymph nodes around the aorta and/or intraperitoneal dissemination.

Among the 12 patients who underwent surgical treatment, intrahepatic recurrence or recurrence at the bile duct stump occurred in five patients (no. 1–3, 5, and 8), and lymph node metastasis occurred in one patient (no. 1). In one (no. 8) of the five patients, solitary intrahepatic recurrent tumor detected at 11 months after the first operation was treated with radiofrequency ablation therapy. Another four (no. 1–3, and 5) patients with recurrence died of carcinoma. Another patient (no. 11) died of hepatic failure. Furthermore, four of the five patients who underwent chemotherapy or conservative treatment died of advanced carcinoma. The median survival time from the diagnosis of cholangiocarcinoma to the death or the end of this study was 578 days.

Discussion

In this study, cholangiocarcinomas were diagnosed in relatively young workers (25–45 years old; mean, 36 years old) in the offset color proof-printing department of a printing company at an extremely high incidence (17 of 111 workers). This type of cholangiocarcinoma is newly classified as an occupational disease by the Ministry of Health, Labour and Welfare, Japan at 1 October 2013.

The peak incidence of cholangiocarcinoma occurred in patients in their sixth or seventh decade and cholangiocarcinoma

is rare in relatively young patients [1–6]. Based on the data from the Osaka Cancer Registry (1975–2007), the mean age at diagnosis for intrahepatic cholangiocarcinoma in male patients ($n = 1797$) was 66.5 ± 0.21 years old and for extrahepatic cholangiocarcinoma in male patients ($n = 3638$) was 68.9 ± 0.19 years old, and the proportions of relatively young patients (25–45 years old) with cancer in intrahepatic and extrahepatic bile duct were 4.1% and 2.6%, respectively [20]. Of 228 patients who were treated for cholangiocarcinoma in the Department of Hepato-Biliary-Pancreatic Surgery in Osaka City University between December 1996 and December 2012 (cholangiocarcinoma in this study was diagnosed during this period), the mean age at diagnosis was 66.2 ± 10.3 years old and the proportion of patients whose age was less than 50 years old was 5.4% (15 patients). Of the 15 patients, five patients were the current or former workers in the printing company. Thus, the mean age of the patients in this study (36 years old) are quite younger, compared to that of typically observed patients with cholangiocarcinoma. Most workers who were exposed to high concentration of chlorinated organic solvent were relatively young, which was related to the age of the patients with cholangiocarcinoma.

Known etiologic factors for cholangiocarcinoma include hepatolithiasis, PSC, pancreaticobiliary maljunction, liver flukes, and exposure to chemical carcinogens, such as nitrosamines [1–4, 7, 8, 16–19], although the causes of most cholangiocarcinoma remain unclear. Other conditions, such as hepatitis B and C viral infections, cirrhosis, alcohol intake, and smoking, are possibly associated with cholangiocarcinoma [8, 21, 22]. Exposure to printing processes, carbon black, and some nitrogenous compounds have been

classified as group 2B (possibly carcinogenic to humans) according to reports by the International Agency for Research on Cancer (IARC) [23]. However, to date, no studies have elucidated the development of cholangiocarcinoma triggered by exposure to printing processes or associated agents. The 17 patients in this study did not exhibit known risk factors for cholangiocarcinoma, although 13 patients were smokers, three were habitual alcohol consumers, and one had a previous history of hepatitis B viral infection. Large amounts of chemicals, including organic solvents, were used in this printing department. Of the 17 patients, all were exposed to 1,2-dichloropropane, 11 were exposed to dichloromethane, and eight were exposed to 1,1,1-trichloroethane. An experiment using reproduction of the working environment by the Japanese National Institute of Occupational Safety and Health suggested that the workers in this department were exposed to high concentration of chlorinated organic solvents [24]. 1,1,1-Trichloroethane, dichloromethane, and 1,2-dichloropropane are classified as category 2 (suspected human carcinogens) according to the Globally Harmonized System of Classification and Labelling of Chemicals [25] and dichloromethane is classified as group 2B according to the IARC [23]. Conversely, other chemicals used in this department were ruled out as possible causative agents because of their lower consumption and/or shorter period of exposure [26]. In addition, other chemicals have been used in various types of industries without inducing cancer. Thus, such chlorinated organic solvents play an important role in the development of cholangiocarcinoma [26]. However, it is impossible to identify all components of the chemicals used previously in the department because the chlorinated organic solvents, such as 1,1,1-trichloroethane, dichloromethane, and 1,2-dichloropropane, have been retired until October 2006, and the organic solvents included impurities. Therefore, other unidentified chemicals might affect the development of the cholangiocarcinoma.

Some cholangiocarcinoma are associated with prior biliary or hepatic disease such as hepatolithiasis, PSC, and liver flukes [2–4, 7, 16–19]. In patients with hepatolithiasis, both BilIN lesions and IPNB are sometimes encountered in the large bile ducts containing stones and in the adjacent bile ducts [10]. PSC is likely an immune mediated, progressive disorder that eventually developed into cirrhosis, and some patients with PSC exhibit only BilIN lesions and not IPNB [18]. In patients with liver fluke infection, bile duct proliferation associated with periportal fibrosis is observed, resulting in cirrhosis [19]. These pathological findings in patients with hepatolithiasis, PSC, or liver flukes indicate neoplastic transformation through dysplastic changes, from bile duct hyperplasia to cholangiocarcinoma (multistep carcinogenesis) [1, 10–12, 18, 19]. In this study, chronic bile duct damage, including duct sclerosis, biliary epithelial

injuries/proliferation, and focal bile duct losses, were observed in various lesions within the resected specimens in all patients for whom operative specimens were available. Furthermore, BilIN-2/3 lesions and IPNB, which are recognized as the premalignant or pre-invasive stages of cholangiocarcinoma, were also observed at various sites of the bile duct and peribiliary glands in all of the patients. The main and most invasive cholangiocarcinoma lesions were located in the large bile duct. These findings indicate that the cholangiocarcinoma detected among the printing company workers might have developed from chronic bile duct injuries into precancerous or early cancerous lesions at various sites of the bile ducts, especially large bile ducts, and eventually developed into invasive cholangiocarcinoma, similar to cholangiocarcinoma in patients with hepatolithiasis, PSC, or liver flukes. In addition, the widespread occurrence of the pathological changes without cirrhosis is an important characteristic of the patients in this study.

Surgery is the only curative treatment for cholangiocarcinoma. In this study, five patients could not be treated surgically because of advanced disease. Thus, to improve patients' long-term outcomes, regular health examinations, including measurements of serum γ -GTP activity and serum CA19-9 and CEA concentrations, are important because the early detection of cholangiocarcinoma is essential. With regard to imaging analyses, both mass lesions with or without dilatation of the peripheral bile duct as well as the dilatation and/or stenosis of the bile ducts are important findings for the detection of cholangiocarcinoma. It is also important to distinguish cholangiocarcinoma from PSC because imaging findings of cholangiography in some patients in this study were similar to those of PSC [14]. It is necessary to monitor the workers for a long time because cholangiocarcinoma was diagnosed at 9 years, 7 months after the stopping of the exposure to the chemicals including chlorinated organic solvent.

In this study, the determination of appropriate treatments was difficult because malignant and premalignant lesions were often observed at various sites in the large bile ducts. In fact, curative resection could not be performed in 5 of the 12 patients who underwent surgery. Adjuvant chemotherapy might be necessary in such patients. Liver transplantation is another treatment option for patients without lymph node involvement or distant metastases.

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for the press (March, 2013) because this event should be publicized to Japanese people to prevent subsequent occurrences. We thank Drs E. Kawamura, S. Marubashi, T. Wakasa, T. Yamamoto, S. Tanaka, and M. Kaji for their assistance with data collection.

Conflict of interest None declared.

Author contribution Study design: SK, YN, TH, and GE designed the study. Acquisition of data: SK, S. Takemura, CS, YU, AN, TN, MK, GH, HT, GT, YM, TY, HT, SN, AA, NK, MF, HF, YS, S. Tanaka, and HT. Clinical aspect of the study: SK, S. Takemura, CS, YU, AN, TN, MK, GH, NK, SU, KKS, TH, and GE analyzed data. SK, YN, NK, KKS, TH, and GE. Pathological aspect of the study: YN, YK, and MO. Data analysis: SK, S. Takemura, CS, YU, AN, TN, MK, GH, NK, SU, KKS, TH, and GE. Manuscript drafted by SK, YN, NK, KKS, TH, and GE. All authors reviewed the manuscript.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 Time course of the patients showing the duration of employment, the time of diagnosis of cholangiocarcinoma, and prognosis. Solid line, the duration of employment with exposure to chlorinated organic solvent; dotted line, the duration of employment without exposure to chlorinated organic solvent; open arrow, diagnosis of cholangiocarcinoma; closed arrow, death.

Table S1 Chemicals used in the offset proof-printing department.

Table S1 Chemicals used in the offset proof-printing department

1,1,1-Trichloroethane	Diethylene glycol monobutyl ether
1,2-Dichloropropane	Propyleneglycol monomethyl ether
Dichloromethane	2-Methyl-2.4pentadiol
Diclorofluoroethane	3-Methyl-3-methoxybutanol
2- Buthanol	Solvent naphtha (coal)
2-Methylpentane	Xylene
3-Methylpentane	Kerosene
N-Hexane	Mineral oil
Cyclohexan	Hydrocarbons
Isopropyl alcohol	Aromatic hydrocarbons
Ethanol	Inks

Figure S1 Time course of the patients showing the duration of employment, the time of diagnosis of cholangiocarcinoma, and prognosis

