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Are hepatolithiasis and cholangiocarcinoma aetiologically related?

A morphological study of 12 cases of hepatolithiasis associated with cholangiocarcinoma

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Summary. A few cases of cholangiocarcinoma (CC) related to hepatolithiasis have been reported to date, but the aetiological relationship remains unclear. In an attempt to clarify the relationship between two phenomena, we examined morphologically 12 cases of hepatolithiasis associated with CC and 26 cases of hepatolithiasis without CC, with the aid of immunohistochemical staining for carcinoembryonic antigen (CEA). In the livers where both hepatolithiasis and CC were found, the carcinoma spread along the lumenal surface of the stone-containing bile ducts and invaded the ductal walls. Features of "chronic proliferative cholangitis" which was a basic feature of hepatolithiasis, were found within the bile duct walls where carcinoma was invading. In some cases of chronic proliferative cholangitis with hepatolithiasis in the absence of CC, atypical epithelial hyperplasia was noted. Atypical epithelial hyperplasia was also found in bile ducts adjacent to and remote from CC. Atypical epithelial hyperplasia was positive for CEA. The data lead us to speculate that chronic proliferative cholangitis in the presence of hepatolithiasis can undergo progressive changes to atypical epithelial hyperplasia which may in turn progress to CC.

Key words: Hepatolithiasis – Chronic proliferative cholangitis – Atypical epithelial hyperplasia – Cholangiocarcinoma

Carcinomas of the extrahepatic biliary tree have been associated with lithiasis especially in the gallbladder (Edmondson 1967; Kuwayti et al. 1957). The morphological features of precursor lesions of the carcinoma related to the lithiasis have been reported (Albores-Saavedra et al. 1980; Esterly and Spicer 1968). In the case of hepatolithiasis, there have been only a few cases reported associated with intrahepatic cholangiocarcinoma (CC) (Falchuk et al. 1976; Kinami et al. 1978; Sanes and MacCallum 1942) possi-

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bly because hepatolithiasis is uncommon in the western hemisphere. The morphological features of hepatolithiasis as a pathological entity have rarely been reported (Yamamoto 1982). In the present study, we described the morphological features of hepatolithiasis and CC related to hepatolithiasis. Further, we identified the putative precursor lesions of CC with the aid of immunohistochemical staining for carcinoembryonic antigen. This study was possible because cases of hepatolithiasis are frequent in Japan compared to the USA and Europe.

Materials and methods

Twelve cases of hepatolithiasis and CC (8 autopsies and 4 surgical liver specimens) and 26 cases of hepatolithiasis without CC (15 autopsies and 11 surgical specimens) were collected from institutions in Japan with the help of the Japanese Hepatolithiasis Study Group (Chairman: Prof. F. Nakayama in Fukuoka). All liver specimens were fixed in 10% formalin and embedded in paraffin. The sections were cut at 4–6 microns thick and stained with haematoxylin-eosin, Azan-Mallory, periodic acid Schiff after diastase digestion (d-PAS) and alcian blue (pH 2.5).

Carcinoembryonic antigen (CEA) was localized by the immunoperoxidase technique described by Sternberger (1979) and Taylor (1976), utilizing deparaffinized sections. Antisera to CEA and peroxidase anti-peroxidase (PAP) complexes were derived from rabbits (Dakopatts, Denmark). Goat antisera to rabbit IgG which was specific for γ-chains were obtained from the Medical Biological Laboratory (Japan). Since Dako's antisera have been known to react with CEA-related substances as well as CEA (Wagner et al. 1978), the antisera were first purified by absorption with normal human liver powder and then perchloric acid extracts from normal lung, meconium and gallbladder bile (Wagner et al. 1978), described below. The absorbed antisera stained carcinoma cells of colon cancer, but did not stain normal colonic epithelium or intrahepatic bile ducts of normal and cirrhotic livers. Negative results were obtained in control sections which had been demonstrated to be positive for CEA when the anti-CEA antisera were replaced by normal rabbit sera and when the anti-CEA antisera were absorbed with the PCA extract from colon cancer.

Tissue extracts were prepared as follows: *Normal human liver powder:* Normal autopsy livers were homogenized in 0.01 M phosphate buffer solution (PBS) (pH 7.4) and filtered through a gauze. Acetone was then added to the residue and the mixture was dried (Migita 1979; Tanaka 1982). *Perchloric acid (PCA) extract from colon cancer:* metastatic nodules of colon cancer (950 g) obtained from a liver of a patient who had extremely high level of serum CEA were homogenized in PBS. An equal volume of 2M PCA was added to the homogenate. The mixture was centrifuged at 4,000 × g for 30 min at 4° and supernatant was dialyzed for 96 h. The residue was filtered through 1.2 μm, 0.45 μm and 0.22 μm membrane filters. The residue was then lyophilized. The residue powder was rich in CEA (Krupey et al. 1972; Tanaka 1982). *PCA extract from normal lung:* lyophilized powder was obtained from human autopsy lungs by the method described above (Mach and Pusztszeri 1972; Tanaka 1982) and this powder was used as a source of normal glycoprotein. *PCA extract from human meconium.* a mixture of normal human meconium (1,050 g) and an equal volume of 1M PCA was centrifuged at 4,000 × g for mins at 4°. Saturated ammonium sulfate was added to the supernatant and the supernatant was centrifuged at 4,000 × g for 30 min at 4°. The resultant powder was dissolved in distilled water and an equal volume of 100% ethanol was added. The resulting mixture was dialyzed and lyophilized. This powder was rich in a non-specific cross reacting antigen-2 (Burtin et al. 1973; Tanaka 1982).

PCA extract from gallbladder bile. Normal gallbladder bile from 6 autopsy cases was centrifuged at 13,000 × g for 1 h at 4° and the supernatant was dialyzed and centrifuged at 28,000 × g for 1 h at 4°. An equal volume of 2M PCA was added to the supernatant. The mixture was then centrifuged at 13,000 × g for 30 min at 4°. Sodium hydroxide solution (2M) was added

to the supernatant and the mixture was dialyzed and then lyophilized. The resultant powder was used as a biliary glycoprotein I (Svenberg 1976; Tanaka 1982).

Results

The clinicopathological features of the patients studied who had hepatolithiasis alone (26 cases) are summarized in Table 1. The main symptoms were abdominal pain, fever and/or jaundice. The age of the patients ranged from 31 to 91 years (mean 61.4 years) and there was a male predominance.

When the livers were examined macroscopically, stones together with sandy bile were found impacted within the dilated lumena of the intrahepatic bile ducts from the hilus of the liver to the subcapsular regions. Distribution of this change was variable in the liver from case to case. Stones were more frequent in the ducts on the left side of the liver when compared with the right. All stones were of the bilirubin calcium type (Kameda 1974; Nagase et al. 1980). Ducts that contained stones and their neighboring ducts which were free of stones appeared dilated in either a cylindrical or saccular configuration. Their walls were thickened by fibrosis. The hepatic parenchyma appeared compressed and atrophic in locations where stones were found in the ducts.

Table 1. Main clinical and pathological data of patients with hepatolithiasis alone and those associated with cholangiocarcinoma

	Hepatolithiasis alone (26 cases)	Hepatolithiasis associated with cholangiocarcioma (12 cases)
Age (mean ± S.D.)	61.4 ± 15.7 years (range: 31–91 years)	55.8 ± 12.3 years (range: 38–80 years)
Sex (% of males)	65.4%	66.7%
Main clinical symptoms		
upper abdomainal pain	76.9% (20/26 cases)	75.0% (9/12 cases)
fever	30.8% (8/26 cases)	41.7% (5/12 cases)
jaundice	23.1% (6/26 cases)	25.0% (3/12) cases)
Period after initial symptoms	30.0 ± 53.8 months (range: 1–240 months)	21.1 ± 23.9 months (range: 2–72 months)
Site of hepatolithiasis		
right lobe alone (%)	11.5% (3 cases)	0%
left lobe alone (%)	53.8% (14 cases)	41.7% (5 cases)
right and left lobes (%)	34.6% (9 cases)	58.3% (7 cases)
Chronic proliferative cholangitis	96.2% (25/26 cases)	80% (8/10 cases)
Atypical epithelial hyperplasia	30.8% (8/26 cases)	80% (8/10 cases)
Association of cholecystolithiasis	53.8% (14/26 cases)	45.5% (5/11 cases)
Association of choledocholithiasis	53.8% (14/16 cases)	45.5% (5/11 cases)

%: percentage of positive cases

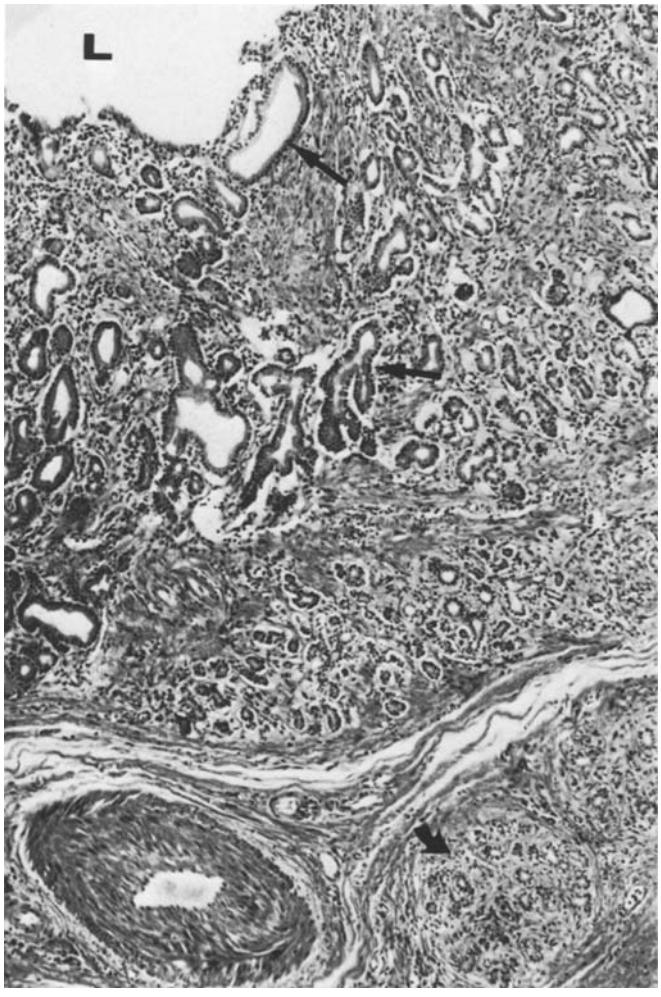

Fig. 1. A microscopic photograph of the wall of a bile duct which contains a stone. The upper half of the picture shows fibrous thickening of the wall with proliferation of glandular elements (*small arrow*) and inflammatory cell infiltrate. In the lower half of the picture the periductal tissue is shown to be fibrotic and acinar cells in a lobular pattern are increased (*curved arrow*). Hepatolithiasis alone. *L:* bile duct lumen. HE., × 90

When the stone containing ducts were examined microscopically, fibrosis of the duct wall and the periductal tissue was prominent (Fig. 1). Lymphocytes, plasma cells, polymorphonuclear and macrophages were consistently present in the infiltrate. Proliferation of glandular elements within the thickened duct walls and also in the periductal connective tissue was a prominent finding. The glandular elements within the thickened duct walls were arranged in a tubular pattern and were mainly composed of mucinous glands which were positive for alcian blue (pH 2.5) and/or d-PAS stains. Also seen were acinar structures surrounded by fibrous tissue in the periductal

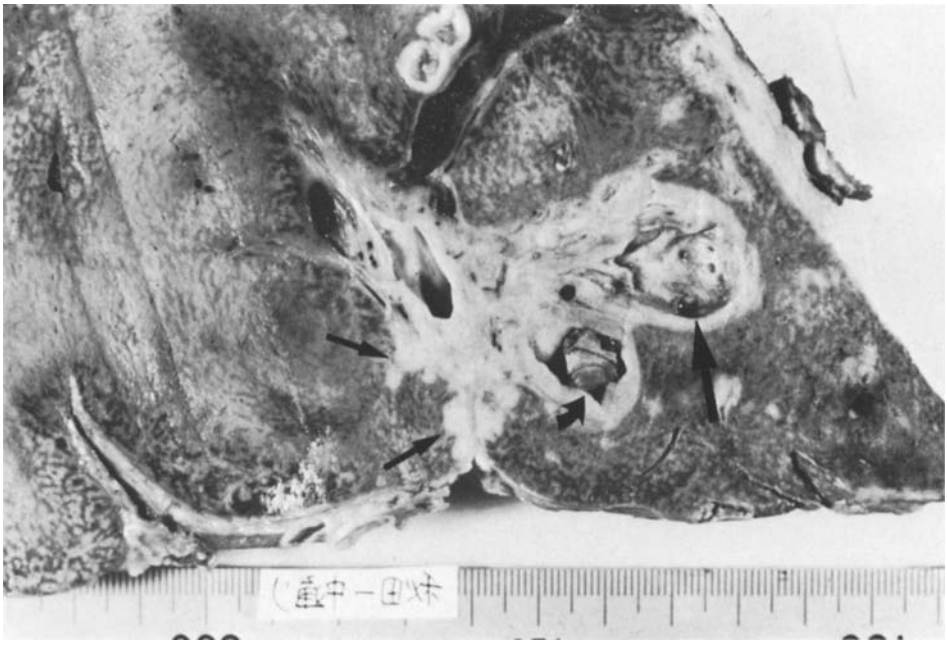

Fig. 2. Gross photograph showing the involvement of stone-filled bile ducts with associated carcinoma (*curved arrow*). Note that the cancer infiltrates the surrounding hepatic parenchyma (*small straight arrow*). The stones have been removed artifically from the bile ducts involved (*big arrow*)

connective tissue. These latter glands were composed of mucinous or serous glands (Fig. 1). The former were positive for alcian blue (pH 2.5) and/or d-PAS, but the latter were negative for both stainings. The mucinous glands resembled the glands found in the normal gastric antrum. The surface lining in the region where stones were located in the ducts was denuded of epithelium. The epithelium still present had a hyperplastic appearance. The ductal lesions described above were found in 25 of the 26 livers and constituted what is referred to in this study as "chronic proliferative cholangitis". The 26th liver revealed a marked granulomatous inflammatory response with a few multinucleated giant cells (granulomatous cholangitis). In the surrounding hepatic parenchyma there was a variable amount of fibrosis but cirrhosis was absent.

The clinicopathological features of the patients with hepatolithiasis and CC (12 cases) are given in Tables 1 and 2. The age and sex distribution, the symptoms and signs, the distribution of stones in the liver and the association of cholecystolithiasis and/or choledocholithiasis were similar to those of the patients who had hepatolithiasis alone (Table 1). The diagnosis of CC was made before death or preoperatively in only 3 cases. The diagnosis was made only on microscopic examination in 4 cases. The main bulk of tumour was found on the left side in 8 cases and on the right side in 3 cases and at the bifurcation of both hepatic lobes in one case (Table 2).

Table 2. Main location and type of carcinoma in the liver, and places of metastasis

Case	Age (years)	Sex	Clinical diagnosis	Main location and type of carcinoma	Metastasis
1(A)	66	female	abdominal tumor	left lobe, periductal spreading type	Lu, Ad, O, Peri, Ly
2(A)	53	male	metastatic liver cancer	left lobe, periductal spreading type	Lu, Peri, Ly
3(A)	49	female	peritonitis carcinomatosa	left lobe, periductal spreading type	Ad, O, Pan, Peri, Ly
4(A)	40	male	cholangio-carcinoma	left lobe, periductal spreading type	Lu, Ad, B, Pan, Peri, Ly
5(A)	47	male	primary biliary cirrhosis	hilus involving both lobes periductal spreading type	–
6(S)	51	male	Caroli's disease	left lobe, periductal spreading type	n.e.
7(S)	59	female	cholangio-carcinoma	left lobe, periductal spreading type	n.e.
8(S)	68	female	hepatolithiasis	left lobe, periductal spreading type	n.e.
0(S)	80	male	hepatolithiasis	left lobe, periductal spreading type	n.e.
10(A)	38	male	acute cholan-gitis	right lobe, massive type	Ad, Ly
11(A)	50	male	bile duct cancer	right lobe, massive type	Ly, Ad, Sp, Kid, Peri, Ly
12(A)	69	male	hepatolithiasis	right lobe, massive type	Ly

Abbreviation. A: autopsy, S: surgical, Lu: lung, Ad: adrenal, O: ovary, Peri: peritoneum, Ly: lymph nodes, Pan: pancreas, Sp: spleen, Kid: kidney, B: bone, n.e.: not examined, –: negative

CC was classified macroscopically as follows: 1. Periductal spreading type: the tumour spread along the intrahepatic stone-containing biliary tree with a variable amount of invasion into the surrounding parenchyma (Fig. 2), and 2. Massive type: the tumour formed a large mass in the right or left hepatic lobe in which stones were embedded in the cancer (Fig. 3). The tumour was a periductal spreading type in 9 cases and a massive type involv-

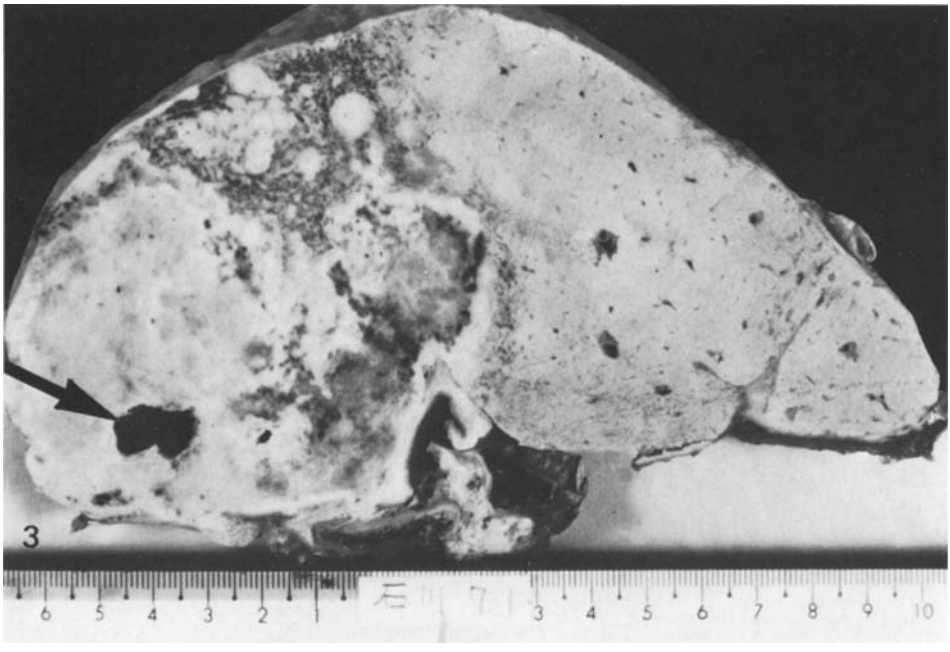

Fig. 3. Gross photograph of right hepatic lobe involved with a large mass of CC with stones (*big arrow*) embedded in the tumour mass which shows necrosis and hemorrhage

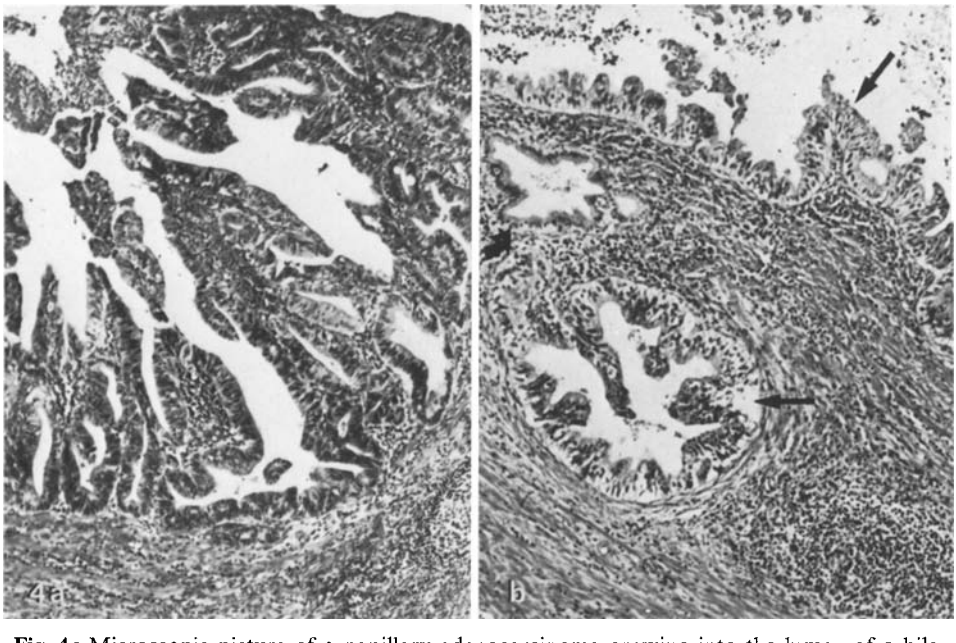

Fig. 4a Microscopic picture of a papillary adenocarcinoma growing into the lumen of a bile duct. Hepatolithiasis with CC. HE., ×90. **b** Microscopic picture of an adenocarcinoma present in the wall of a stone containing bile duct. A micropapillary pattern is seen (*small arrow*). A non-cancerous tubular gland (*curved arrow*) is seen in the duct wall. Hepatolithiasis with CC. HE., ×90

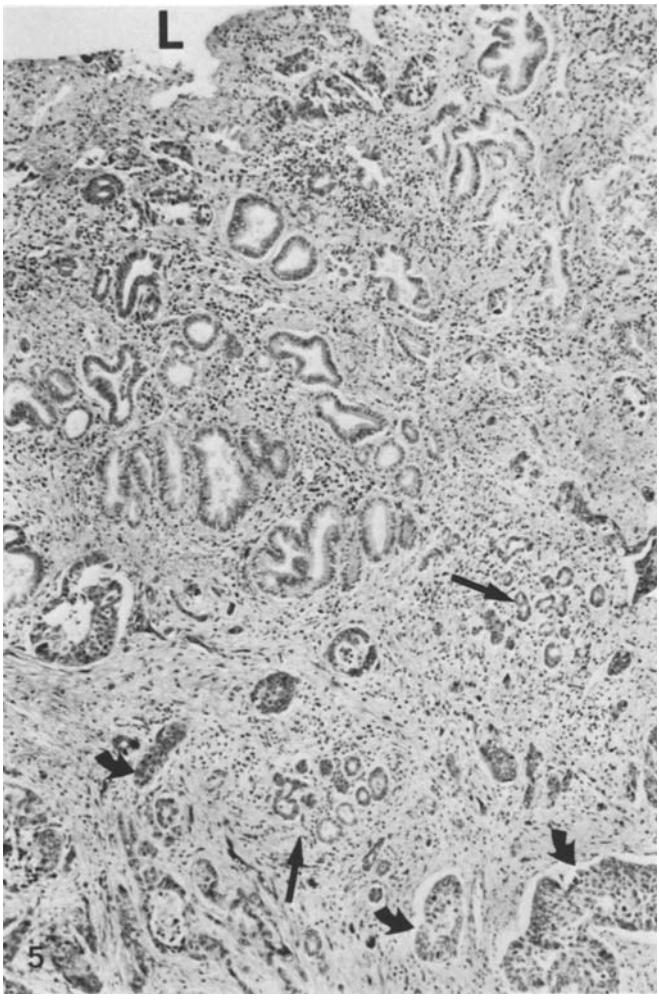

Fig. 5. Microscopic picture of a bile duct containing carcinoma with associated stones. There is a well differentiated tubular adenocarcinoma which infiltrates an area of pre-existing chronic proliferative cholangitis (*small arrow*). A solid pattern (*curved arrow*) of carcinoma can be seen in the lower part of the picture. *L* bile duct lumen. HE., ×90

ing mainly the right lobe in 3 cases. It was grossly grayish white and firm and necrosis and hemorrhage were frequent, especially in the massive type. Stones were found in intrahepatic bile ducts remote from the CC in 3 of the 12 cases. Associated with the stones was chronic proliferative cholangitis as seen in hepatolithiasis without CC. The atrophy of the hepatic parenchyma seemed more advanced in hepatolithiasis with CC than in hepatolithiasis without CC in areas harboring stones.

In all 12 cases of CC, the pattern was that of adenocarcinoma. One case of periductal spreading type showed papillary adenocarcinoma with scanty infiltration into the duct wall (Fig. 4a). The remaining 8 periductal

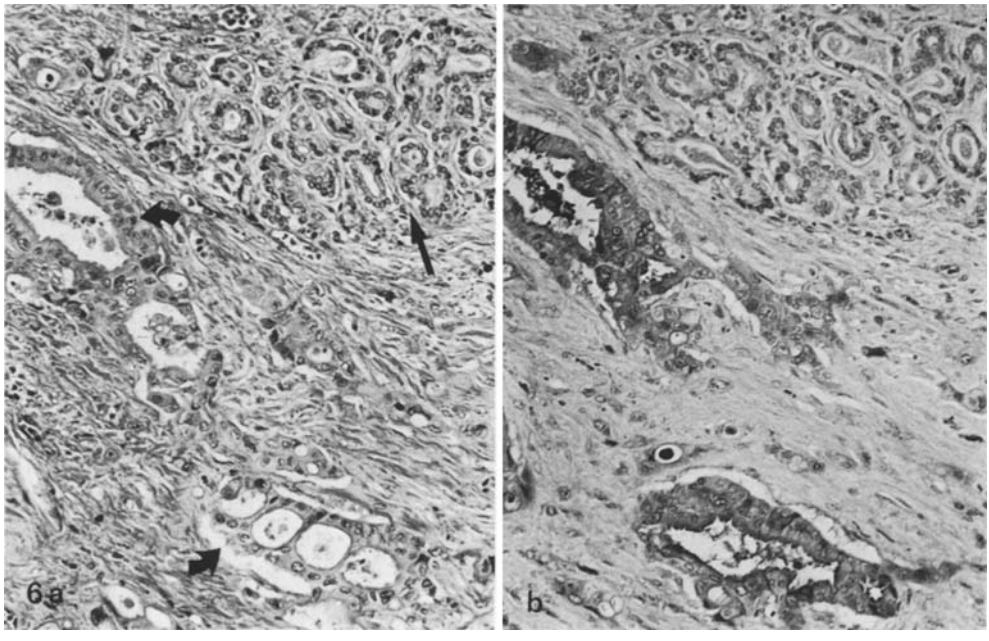

Fig. 6. **a** Chronic proliferative cholangitis is seen microscopically (*small arrow*) mixed with infiltrating adenocarcinoma (*curved arrow*) in a duct containing stones. HE., ×190. **b** Photomicrograph of a stain for CEA showing reaction product present in the cytoplasm and in the secretory products of an adenocarcinoma. A non-neoplastic component at the top of the photograph consisting of chronic proliferative cholangitis does not contain CEA. PAP stain for CEA with haematoxylin counter-stain, ×190. **a** and **b** are step serial sections of the same surgical specimen

spreading type carcinomas were micropapillary and tubular in architecture. The cancer in these 8 cases spread along the lumenal surface and invaded the ductal wall and periductal tissue (Figs. 4b and 5). In all the cases of massive cancer surrounding stones, there was extensive necrosis present so that histological features of the original duct that contained the stones were obscure. Where the tumour had invaded into the surrounding hepatic parenchyma, the papillary and tubular differentiation was lost and it grew in a solid pattern with accompanying desmoplastic response (Fig. 5). Perineural invasion was common. No cases showed squamous elements, large cystic cavity formation or signet ring cell differentiation. CEA and/or mucin were found in the cytoplasm, on the lumenal border of the cancer cells and in the secretory products of the carcinoma cells in all cases examined (Fig. 6).

Chronic proliferative cholangitis was seen in the bile ducts which were located adjacent to the CC. Differentiated glands which had no cytological features of malignancy were seen mixed here and there in the bile duct walls involved by carcinoma in 8 of the 9 cases of the periductal spreading type (Figs. 5 and 6). These differentiated glands were negative for CEA making it possible to distinguish them from the associated carcinoma which showed the presence of positive reaction products (Fig. 6b).

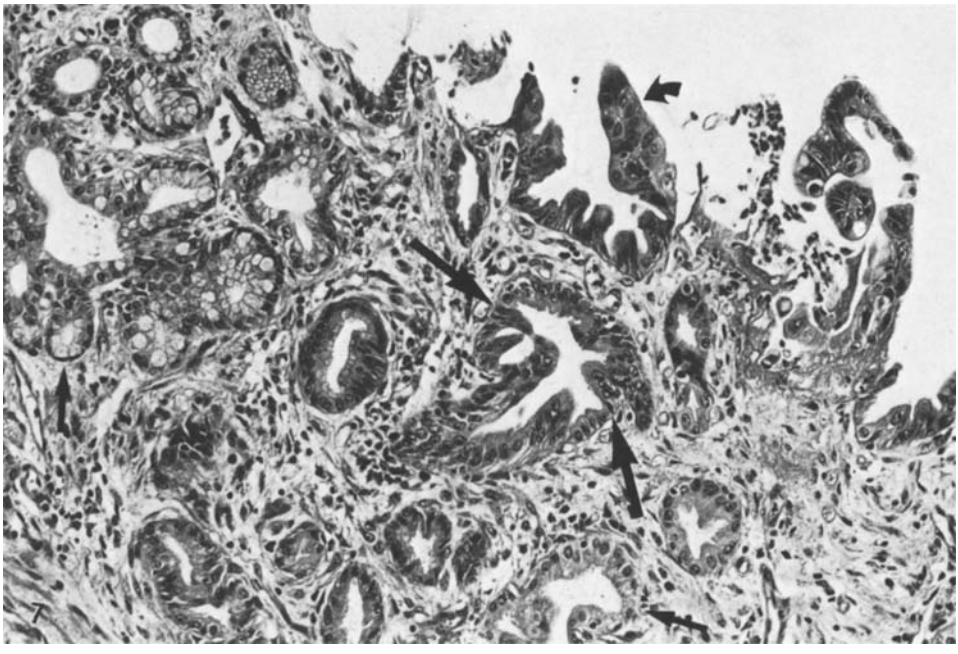

Fig. 7. Microscopic photography of atypical epithelial hyperplasia is shown. The hyperplastic surface layer of cells (*curved arrow*) and the proliferating glands within the wall (*big arrow*) can be distinguished. Glandular elements of chronic proliferating glands which do not show atypical changes are noted (*small arrow*). Hepatolithiasis alone. HE., ×190

Atypical epithelial hyperplasia was noted associated with chronic proliferative cholangitis. The cells lining the lumen and the glandular epithelium in the chronic proliferative cholangitis appeared hyperplastic and showed features such as stratification, nuclear hyperchromasia and abnormally tall columnar cell change. In addition, nuclear atypism was occasionally noted where there were partial loss of polarity, increase in the nuclear-cytoplasmic ratio and a few mitotic figures (Figs. 7 and 8). Some of the atypical glands showed micropapillary or villous structure formation (Fig. 8). These lesions were termed "atypical epithelial hyperplasia" in this study. This change was usually seen in only one to several foci in the stone-bearing bile ducts. The atypical epithelial hyperplasia was found in 8 of the 26 livers with hepatolithiasis alone and in the stone-containing ducts adjacent to and/or remote from the carcinoma of 8 of the 10 livers with CC examined. Occasionally it was difficult to distinguish with certainty the difference between atypical hyperplasia and carcinoma in situ. CEA was occasionally detected in the atypical hyperplastic epithelia along the cell borders of the glandular lumena (Fig. 8b). This feature was not found in the ductal and glandular epithelia which did not exhibit atypia.

Discussion

A study conducted by Kusama (1978) disclosed that the total number of patients with hepatolithiasis was approximately 1,500 in Japan in the period

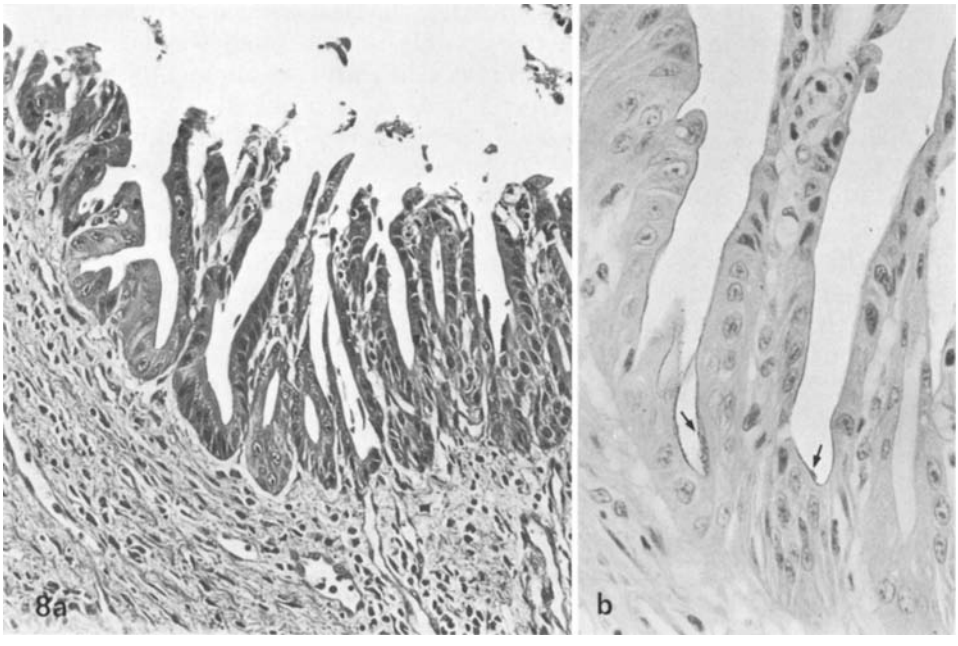

Fig. 8a Microscopic picture of atypical biliary epithelium with villous pattern is shown. These cells are pleomorphic and have nuclear hyperchromasia. HE., ×190. **b** Section taken from **a** and stained with CEA antisera shows reaction product on the luminal surface of the atypical biliary epithelium (*small arrow*). **a** and **b** are step serial sections of the same surgical specimen from a patient with hepatolithiasis without *CC*. PAP stain for CEA and hematoxylin, ×375

from 1966 to 1978. It is generally said in Japan that about 10% of patients with hepatolithiasis have associated CC (Kinami et al. 1978). However, an exact incidence has not been determined by epidemiological studies. In the present study, we found that the main clinicopathological features of hepatolithiasis did not differ whether or not CC was associated. For this reason, a careful examination of all patients with hepatolithiasis is necessary in order to detect CC. Further, surgical pathologists must be aware of the association and look carefully for CC in surgically resected specimens by taking numerous tissue blocks for sectioning because a small CC is occasionally detected in a portion of the duct that contains a stone.

The following findings obtained in the present study seem support a positive aetiological relationship between hepatolithiasis and CC. First, in all cases examined, stones were found embedded or impacted within cancer tissue or within ducts involved with cancer, suggesting an intimate topographical relation between stones and cancer. Second, we noted the existence of "chronic proliferative cholangitis" in ducts containing stones in cases without CC. The ducts that were involved with cancer still retained features of chronic proliferative cholangitis so that a mixture of non-cancerous proliferative glands and cancer cells was found in the wall of affected ducts. We interpreted this to mean that chronic proliferative cholangitis preceded the development of CC. Third, putative precursor lesions of CC were also

established in this study of hepatolithiasis. That is, we noted atypical epithelial hyperplasia in case where hepatolithiasis was found whether or not there was an associated CC. There was difficulty discriminating between in situ carcinoma and severe atypical hyperplasia in the walls of ducts that contained stones. These changes have not been clearly described to date.

Albores-Saavedra et al. (1980) reported in chronic cholecystolithiasis that a small number of hyperplasia of the gallbladder occur and evolve toward atypical hyperplasia and that this progresses to in situ carcinoma which finally becomes invasive carcinoma. Extrapolation of these sequences in chronic cholecystolithiasis and the results obtained in the present study lead us to a hypothesis that intrahepatic stones formation leads to carcinoma development through a series or sequences of events as follows: chronic proliferative cholangitis with epithelial hyperplasia is followed by atypical hyperplasia which then progresses to neoplastic change. Atypical or adenomatous hyperplasia of the duct epithelium has been described previously as a precancerous lesion associated with CC in diseases such as Caroli's disease (Gallagher et al. 1972; Jones and Shreeve 1970; Phinney et al. 1981) and *Chlonorchis sinensis* infection (Hou 1956). Thus, hepatolithiasis should be added to Caroli's disease and *Chlonorchis sinensis* infection as a precancerous condition for CC. In this study, we noted that CEA staining was positive in CC. Antisera to CEA react with a family of glycoproteins which can be found in a variety of normal and diseased tissue and neoplasm (Gerber and Thung 1978; Gerber et al. 1983). However, our antisera to CEA reacted more specifically with colon carcinoma cells after several absorptions by CEA-related substances. Thus, in our study, we successfully detected atypical epithelia and carcinoma cells without staining normal or hyperplastic duct epithelium using our absorbed antisera. This allowed us to speculate that the atypical hyperplasia is a potentially neoplastic lesion since it was also stained with our antisera to CEA.

We postulate that the atypical hyperplasia and CC in chronic proliferative *Cholangitis* result from repeated ulceration and recurrent repair of the duct lining and underlying glandular epithelium caused by stone erosion similar to what is seen in other organs (Grundmann 1983). This sequence was described by Hou (1956 and 1964) in cats infested with *Clonorchis sinensis* within the intrahepatic bile duct system. Also, it is possible that other factors such as bacterial products from repeated infections, the presence of secondary bile acids, the persistence of inflammatory change and the presence of physical and chemical irritation as stones grow within the ducts might contribute to activation of procarcinogens or act as promoting factors for carcinogenesis (Connor et al. 1979; Goldin and Gorbach 1983; Reddy and Watanabe 1979). A prospective long-term follow-up study involving the patients with hepatolithiasis will be necessary to resolve the question proposed by our study, that is, whether or not intrahepatic stones have a predisposition to the development of CC.

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