Histologic Features and Bile Duct Lesions in the Alcoholic

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Histologic features of 112 liver biopsy specimens from patients with alcoholic liver injury were studied. Alcoholic hyalin was found in 6 specimens (5.4%). Spherical giant mitochondria were detected in 9 specimens (8.0%). Fatty metamorphosis, infiltration by polymorphonuclear leucocytes, and pericellular fibrosis were observed in many specimens. Zonal or submassive necrosis was also found in 20 specimens (17.9%). Cholestasis was seen in 32 specimens (28.6%). Histologic changes in interlobular bile ducts were investigated in 103 specimens containing such ducts. Among 31 specimens with cholestasis, changes in interlobular bile ducts were detected in 19 specimens (61.3%), whereas biopsy specimens without cholestasis showed the changes in only 4 of 72 (5.6%). Changes observed in interlobular bile ducts included inflammatory cell infiltration and vacuolation of epithelium in most specimens. Swollen or flattened epithelial cells, karyopyknosis and eosinophilic degeneration were observed in many. These results suggest that the changes of interlobular bile ducts may be partially related to cholestasis in alcoholic liver injury.

Key Words: Alcoholic liver injury, Cholestasis, Interlobular bile duct

In Japanese alcoholics, cirrhosis, hepatic fibrosis, alcoholic hepatitis, fatty liver and non-specific minimal changes have been recorded.

Individual histologic features described¹⁾ include alcoholic hyalin, fatty infiltration, hemosiderosis, pericellular fibrosis, polymorphonuclear leucocyte infiltration and giant mitochondria, among others. Moreover cholestasis is occasionally seen and it has been suggested that cholestasis may play some role in the progression of liver damage²⁾ and portal-tract changes may morphologically mimic those of bile duct obstruction³⁾. Experimental alcoholic liver injury has revealed that cholestasis can appear from an early stage and it has been postulated that cholestasis is partially related to fibrosis⁴⁾. Afshani et al.⁵⁾ have showed microscopic cholangitis in

alcoholic liver injury with severe cholestasis, and concluded that microscopic cholangitis was a feature of severe cholestasis which may accompany alcoholic liver injury.

The authors have investigated the histologic features in the liver in Japanese alcoholics and have attempted to elucidate the relationship between cholestasis and changes in interlobular bile ducts.

MATERIALS AND METHODS

The material consisted of 112 liver needle biopsy specimens obtained from 106 patients with a history of excessive drinking of alcoholic beverages (more than 80 g ethanol daily) for over 10 years.

The biopsy specimens were fixed in 10%

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formalin and embedded in paraffin. All biopsy specimens were cut at 4 μ thickness. Hematoxylin and eosin (H&E), azan-Mallory, Gomori reticulin, periodic acid-Schiff stains after diastase digestion, Orcein and Masson's trichrome stains were carried out.

Of the 112 specimens, 103 contained interlobular bile ducts from 98 patients were selected for investigating the changes of interlobular bile ducts.

A diagnosis of alcoholic hepatitis was based on morphologic criteria⁶⁾. A diagnosis of hepatic fibrosis was based on the description by Wepler⁷⁾. The cholestasis was used here in its morphologic sense⁸⁾.

All patients had clinically and/or histologically no evidence of viral hepatitis, drug induced hepatic injury and cholelithiasis.

Statistical analyses were done by χ^2 test.

RESULTS

1) Histological diagnosis

Among 112 specimens from 106 patients diagnosed clinically and/or histologically as alcoholic liver injury, histological diagnoses were hepatic cirrhosis in 11 specimens (all specimens without alcoholic hepatitis), hepatic fibrosis in 55, alcoholic hepatitis in 6, fatty liver in 12, nonspecific changes in 23 and others in 5, re-

spectively.

2) Histologic features

Incidence of various histologic changes is shown in Table 1. Alcoholic hyalin was found in 6 specimens (5.4%); one of 55 with hepatic fibrosis and 5 of 6 with alcoholic hepatitis. Spherical giant mitochondria were seen in 9 specimens (8.0%); 4 with hepatic fibrosis, one with alcoholic hepatitis, 3 of 12 with fatty liver and one other. Various degree of fatty metamorphosis were found in 66.1%. Polymorphonuclear leucocyte infiltration was observed in all 6 specimens of alcoholic hepatitis and variously found in 56.6% of biopsies with other histological diagnoses. Pericellular fibrosis in the periportal and central areas of lobules was seen in most specimens of hepatic cirrhosis, hepatic fibrosis and alcoholic hepatitis, and was seen to a slight degree in 62.5% of biopsies with other histological diagnoses. Zonal or submassive necrosis was also observed in 20 specimens; 9 with cirrhosis, 7 with hepatic fibrosis, 3 with alcoholic hepatitis and 1 other. Cholestasis was seen in 32 specimens (28.6%); 3 with cirrhosis, 20 with fibrosis, 3 with non-specific changes and 1 other.

3) Clinical findings (Table 2)

Of the 112 specimens, 103 contained interlobular bile ducts from 98 patients were investigated.

Table 1. Histologic features

No. of specimens		Hepatic cirrhosis 11	Hepatic fibrosis 55	Alcoholic hepatitis 6	Fatty liver 12	Nonspecific changes 23	Others 5	Total 112
Alcoholic hyalin	- +	11 0	54 1	1 5	12 0	23 0	5 0	106 6
Giant mitochondria	- +	11 0	51 4	5 1	9 3	23 0	4 1	103 9
Fatty metamorphosis	- + ++ +++	3 8 0 0	19 28 6 2	0 3 3 0	0 0 9 3	14 9 0 0	2 2 1 0	38 50 19 5
Polymorpho- nuclear cell infiltration	- + ++ +++	7 1 3 0	26 27 2 0	0 3 2 1	5 7 0 0	7 16 0 0	1 3 1 0	46 57 8 1
Pericellular fibrosis	- + ++ +++	1 1 6 3	0 4 21 30	0 2 2 2	5 7 0 0	8 15 0 0	2 3 0 0	16 32 29 35
Zonal or submassive necrosis	- +	2 9	48 7	3 3	12 0	23 0	4 1	92 20
Cholestasis	- + ++	8 2 1	35 17 3	3 3 0	10 2 0	20 3 0	4 1 0	80 28 4

Table 2. Clinical findings

		Alcoholic liver injury (98 cases)	
		(with cholestasis)	(without cholestasis)
		31 specimens obtained from 29 cases (Group A)	72 specimens obtained from 69 cases (Group B)
Sex	male	27	63
	female	2	6
Age (years) (mean $\pm$ SD)		47 $\pm$ 10	45 $\pm$ 10
Duration of jaundice	(-)	9/29	55/69
	~1week	6/29	5/69
	1-4weeks	8/29	6/69
	4weeks~	6/29	3/69
		(mean $\pm$ SD)	(mean $\pm$ SD)
Total bilirubin (mg/dl)		4.4 $\pm$ 4.7 ***	1.6 $\pm$ 1.9
GOT		77.8 $\pm$ 146.4 *	40.9 $\pm$ 39.2
GPT		50.2 $\pm$ 70.0	45.0 $\pm$ 36.4
Alkaline phosphatase (K.A.)		13.0 $\pm$ 9.9 ***	8.1 $\pm$ 3.1
γ -GTP (mU/ml)		244.0 $\pm$ 323.2 **	124.1 $\pm$ 127.1
γ -Globulin (g/dl)		1.48 $\pm$ 0.45 **	1.21 $\pm$ 0.46
ICG retention in 15 min.		18.4 $\pm$ 17.6	14.9 $\pm$ 21.9

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

At the time of biopsies cholestasis was seen in 31 specimens from 29 patients (Group A) and it was not seen in 72 specimens from 63 patients (Group B). There were 27 males and 2 females in Group A. Sixtythree males and 6 females were in Group B. The average age was 47 in Group A and 45 in B. Jaundice was not found in 9 of Group A and in 55 of Group B. In Group A the duration of jaundice was less than one week in 6, from one to 4 weeks in 8, more than 4 weeks in 6. In Group B the duration of jaundice was less than one week in 5, from one to 4 weeks in 6, more than 4 weeks in 3.

Comparison of laboratory findings at the time of biopsies between Group A and Group B is also shown in Table 2. Total bilirubin, GOT, alkaline phosphatase, γ -GTP and γ -Globulin were significantly higher in Group A than in Group B.

4) Bile duct lesions

The incidence of changes in interlobular bile ducts in specimens with and without cholestasis is given in Table 3. Among 31 specimens with cholestasis, various changes of interlobular bile ducts were observed in 19 specimens (61.3%); 2 of 3 specimens diagnosed microscopically as hepatic cirrhosis, 12 of 19 specimens diagnosed

Table 3. Incidence of interlobular bile duct changes

Histological diagnosis	Alcoholic liver injury	
	(with cholestasis)	(without cholestasis)
Hepatic cirrhosis	2/3	0/7
Hepatic fibrosis	12/19	2/32
Alcoholic hepatitis	2/3	0/3
Fatty liver	1/1	0/9
Nonspecific changes	2/4	2/19
Others	0/1	0/2
Total	19/31	4/72

 $p < 0.001$

Fig. 1-A. An interlobular bile duct showing moderate changes is seen in the center of the field. Eosinophilic degeneration of some epithelial cells with karyopyknosis is noted. Inflammatory cells are also found in the epithelium. The lumen contains cellular debris. (H&E, $\times 400$)

Table 4. Incidence of histologic changes of interlobular bile ducts

No. of specimens	Alcoholic liver injury		Total
	(with cholestasis) 19	(without cholestasis) 4	
Cell swelling and/or flattening	6	0	6
Karyopyknosis	3	0	3
Eosinophilic degeneration	4	2	6
Vacuolated cytoplasm	11	2	13
Inflammatory cells in the epithelium	19	4	23
Pseudo-stratification	0	0	0
Lumen Obstruction	3	0	3
Cellular debris	11	3	14
Rupture of the wall	0	0	0

as hepatic fibrosis, 2 of 3 specimens diagnosed as alcoholic hepatitis, one of the specimens diagnosed as fatty liver, and 2 of 4 specimens showing nonspecific changes. Among 72 specimens without cholestasis, however, changes in interlobular bile ducts were found in only 4 specimens (5.6%); 2 of 32 specimens diagnosed as hepatic fibrosis and 2 of 19 specimens with nonspecific changes. There was a significant difference in the frequency of the changes of interlobular bile ducts between these two groups ($p < 0.001$).

The incidence of various histologic changes of

Fig. 1-B. The same biopsy specimen found in Fig. 1-A. Pericellular fibrosis is observed in central area. (Gomori reticulin, $\times 100$)
Histological diagnosis: Hepatic fibrosis

interlobular bile ducts is set out in Table 4. The changes observed in interlobular bile ducts comprised inflammatory cell infiltration in the epithelium and epithelial vacuolation in most specimens (Figs. 1-A, 2-C, 3-A). Swollen or flattened epithelial cells, karyopyknosis and eosinophilic degeneration (Fig. 3-A) were observed in many specimens. Cellular debris was often found in the lumen (Fig. 1-A). Neutrophils in the lumen were seen in only one specimen with cholestasis. The lumen was obstructed by swollen epithelial cells,

Fig. 2-A. Ballooning of liver cells and liver cell necrosis with polymorphonuclear infiltrate are seen. (H&E, $\times 200$)

Histological diagnosis: Alcoholic hepatitis

Fig. 2-B. The same specimen found in Fig. 2-A. An affected bile duct (arrow) is shown in the center of the figure. (H&E, $\times 200$)

Fig. 2-C. Higher magnification of Fig. 2-B. The epithelium is infiltrated by inflammatory cells and some epithelial cells have vacuolated cytoplasm. The lumen contains cellular debris. (H&E, $\times 1000$)

bile thrombi or cellular debris in 3 specimens. Pseudostratification of epithelial cells and duct rupture were not observed. In portal tracts containing affected interlobular bile ducts, mild inflammation and ductular proliferation were found. Follicle formation and granuloma were not observed.

DISCUSSION

There have been many reports on the morphological features of hepatic injury in alcoholics. Various acute histologic changes superimposed on chronic liver injury probably follow excessive

drinking, except where such changes are minimal. Alcoholic hyalin, polymorphonuclear leucocyte infiltration, giant mitochondria and cholestasis may be superimposed on fatty liver, hepatic fibrosis and hepatic cirrhosis to a variable degree.

Alcoholic hyalin is one of the most important changes for the histological diagnosis of alcoholic hepatitis⁶. However, low incidence of alcoholic hyalin has been described in Japan^{1, 9, 10}. Indeed, alcoholic hyalin was found in only 5.4% of our material.

Recently it has been reported that giant mitochondria were detected by light microscopy in

Fig. 3-A. Swollen or flattened epithelial cells, karyopyknosis and eosinophilic degeneration are observed. The lumen contains bile thrombi. (H&E, $\times 1000$)

Fig. 3-B. The same specimen shown in Fig. 3-A. Portal and pericellular fibrosis are noticeable.

(Gomori reticulin, $\times 100$)

Histological diagnosis: Hepatic fibrosis

a high proportion of alcoholics and rarely in non-alcoholic liver diseases. Bruguera et al.¹¹⁾ reported that the incidence of giant mitochondria was significantly higher in patients with high alcohol consumption (72%) than in those with low or no alcohol intake (10%), and that their presence was related to the amount of daily ethanol consumption and to the shortness of abstinence before the biopsy. Consequently they emphasized that megamitochondria should be considered as a diagnostic clue of recent and heavy alcoholism. However, in our study only 9 specimens had giant mitochondria in 112 specimens obtained from patients

who drank more than 80 g of ethanol daily for over 10 years.

In alcoholic hepatitis, liver cell necrosis with polymorphonuclear infiltration, usually but not always accompanied by steatosis, is an important findings⁶⁾. In this study, polymorphonuclear leucocyte infiltration and fatty change were found in all specimens diagnosed as alcoholic hepatitis, and in many specimens with other histological diagnoses.

In the liver of Japanese alcoholics fibrosis of portal tracts extending into the lobules pericellularly has been emphasized as an important factor in transition to cirrhosis¹²⁾. The authors also found pericellular fibrosis^{3, 13)} in the periportal and central areas of lobules in almost all biopsy specimens diagnosed as hepatic cirrhosis, hepatic fibrosis and alcoholic hepatitis. Moreover minor degrees of pericellular fibrosis were detected in 60% of specimens diagnosed as fatty liver and those showing nonspecific changes.

Zonal and/or submassive necrosis was observed in 9 of 11 specimens diagnosed as cirrhosis, 7 of 55 with hepatic fibrosis and 3 of 6 with alcoholic hepatitis. This necrosis may play the important role in the progression of hepatic injury, and hepatitis virus may be implicated in such necrosis in Japanese alcoholics¹⁾.

Cholestasis is also occasionally found in alco-

holic liver injury. We found mostly mild cholestasis in 28.6% of specimens from patients with alcoholic liver injury. Afshani et al.⁵⁾ studied liver biopsy specimens from 23 consecutive alcoholic patients who had a convincing history of excessive alcohol intake and hyperbilirubinemia of more than 1.5 mg/dl. Patients with polymorphonuclear leucocytes in multiple bile ducts (microscopic cholangitis) had significantly higher serum SGOT, bilirubin, alkaline phosphatase and cholesterol than those with no or rare intra-ductal polymorphonuclear leucocytes. Histologically, periductal acute inflammation and severe panlobular cholestasis were more often found in the former group. The significance of microscopic cholangitis was emphasized in the severe cholestasis which may accompany alcoholic liver injury. In this study, changes observed in bile ducts included inflammatory cell infiltration, vacuolation of epithelium, swollen or flattened epithelial cells and others. Our findings were not always similar to the features of microscopic cholangitis characterized by polymorphonuclear leucocytes infiltration. These difference may be due to severity of cholestasis. Cholestasis observed in our series were mostly mild.

Intrahepatic bile ducts are affected in primary biliary cirrhosis¹⁴⁾, in viral hepatitis¹⁵⁻¹⁷⁾, in some drug induced hepatic injury^{18, 19)}, in graft-versus-host disease²⁰⁾ and in others²¹⁾. Epithelial changes of interlobular bile duct observed in alcoholics were not so destructive as those in primary biliary cirrhosis¹⁴⁾ and similar to epithelial changes in viral hepatitis¹⁷⁾. The portal tracts were markedly infiltrated by inflammatory cells and follicle formation was occasionally found in viral hepatitis. In alcoholic liver injury, however, inflammation in portal tract was mild and follicle formation was not seen. In our series, histologic changes of interlobular bile ducts were more often found in liver biopsy specimens with cholestasis than in those without. This findings suggests that the changes of interlobular bile ducts seem to be related to cholestasis in alcoholic liver injury. Recently, it was reported that pancreatitis might complicate the picture in alcoholics with portal changes as in large duct obstruction and with parenchymal cholestasis^{22, 23)}. Bile duct changes in the alcoholic

may be able to be explained by such mechanism. Further investigation is needed to establish whether the interlobular bile ducts are directly injured or not by alcohol.

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