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Intraparenchymal Isolated Bile Ductules in the Needle Biopsy Specimens of the Liver: An Interobserver Variation and Clinicopathological Study

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Abstract

Intraparenchymal isolated bile ductules (IIBDs) are occasionally recognizable in liver specimens, but their characteristics remain poorly understood. Therefore, we (2 pathologists) attempted to identify IIBDs in 81 hematoxylin and eosin-stained needle biopsy specimens of the liver, and examined the interobserver variation and their clinicopathologic features. Aberrant cytokeratin 7 (CK7) expressions of hepatocytes, known to represent hepatic progenitor cell (HPC) activation, were also evaluated. In each specimen, the mean number of IIBDs counted by us was divided by the number of portal areas, which was defined as the IIBD score. Both pathologists detected IIBDs in 53 specimens (65.4%), indicating that they are not rare. Observed agreement was obtained in 81.5%, and the kappa statistic indicated moderate agreement (kappa: 0.515). Higher IIBD score was closely associated with a higher degree of hepatocytic CK7 expression in all areas (P=0.012), periportal areas (P=0.007), and peri-centrilobular vein (CV) areas (P=0.032), but not with age, gender, increased serum levels of liver dysfunction tests, or hepatic steatosis. Ductular reaction was also associated with hepatocytic CK7 expression in all areas (P=0.004), periportal areas (P=0.024), and peri-CV areas (P=0.031). However, there was not a relevant relationship between higher IIBD score and a severe degree of ductular reaction. In non-alcoholic steatohepatitis (NASH) cases, significant IIBD score was correlated with grading score (P=0.043), but not with fibrosis staging score. In viral hepatitis cases, it was not associated with grading or staging score. These results suggested that the development of IIBD can highlight HPC activation, but might be independent of periportal HPCs, also known as “periportal niches”. The presence of IIBDs would be due in part to a hepatic necro-inflammatory state in some conditions, such as NASH.

Keywords: Isolated bile ductile; Cholangiocyte; Liver; Ductular reaction; Cytokeratin 7

Abbreviations

IIBD: Intraperenchymal Isolated Bile Ductule; ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; CK: Cytokeratin; CV: Centrilobular Vein; DR: Ductular Reaction; H&E: Hematoxylin and Eosin; LDH: Lactate Dehydrogenase; NASH: Nonalcoholic Steatohepatitis; Γ-GT: Γ-Glutamyltransferase

Introduction

The small terminal bile ducts or the finer branches of the biliary tree can be divided into the canals of Hering and bile ductules. The former are lined by both hepatocytes and cholangiocytes, connecting to bile canaliculi [1-5]. The latter are short fine tubules with lumina less than 15-20 micrometers entirely lined by 2 to 6 cuboidal cholangiocytes, and extend through the limiting plate, connecting to interlobular bile ducts in the portal tracts [1-3]. Intraparenchymal bile ductules are hardly visible in a normal liver [5], but, in various cholestatic conditions, periportal parenchyma and portal areas can be involved by proliferating ductules in anastomosing networks accompanied by an inflammatory infiltrate and fibrosis, referred to as ductular reaction (DR) [3,5]. On the other hand, we have occasionally encountered intraparenchymal isolated bile ductules (IIBDs) without typical anastomosing features of ductular reaction. To our knowledge, the clinicopathologic significance of such IIBDs remains poorly understood, although a recent article has described similar IIBDs in chemotherapy-induced liver injury [6]. In this study, we attempted to identify IIBDs in needle biopsy specimens of the liver, and examined their clinicopathologic characteristics. The interobserver variation in recognizing IIBDs was also investigated.

Materials and Methods

A total of 81 needle biopsy specimens of the liver, retrieved from the surgical pathology files of the Department of Pathology, Japan Self-Defense Forces Central Hospital (2005-2010), were examined. Liver specimens had at least 4 centrilobular veins (CVs) (range, 4-15; mean, 8.9) and 6 portal areas (range, 6-16; mean, 10.4). They were removed from 81 patients (70 men and 11 women) with a mean age of 44.1 years (range, 19-68 years), including 33 patients with hepatitis C virus infection only, 21 with hepatitis B virus infection only, 14 with nonalcoholic steatohepatitis (NASH) only, and 13 with alcoholic liver diseases only. Clinical information was obtained from the patients' charts and/or the request forms of pathologic examination. In all patients, serum levels of total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and γ-glutamyltransferase (γ-GT) were obtainable; the time interval between the serum examination and the

liver biopsy ranged from 0 to 9 days (mean, 1.15 days). All paraffin-embedded specimens were available, and additionally cut, stained with hematoxylin and eosin (H&E), and immunostained with CK7 (OV-TL 12/30; Dako, Glostrup, Denmark).

On the basis of H&E-stained histology, identification of IIBDs was attempted independently by 2 pathologists (S.M. and K.S.), and the interobserver variation was evaluated using kappa statistic [7]. In each specimen, the mean number of IIBDs counted by 2 pathologists was calculated, and was divided by the number of portal areas, which was defined as "IIBD score". On the basis of the mean number of this score, we defined cases in which such IIBD score was more than 0.28 as being "significant." Hepatic steatosis was graded as follows: 0, <5% of hepatocytes affected; 1, 5-33% of hepatocytes affected; 2, 34-66% of hepatocytes affected; or 3, >66% of hepatocytes affected. Using a modified version of the method of Eleazar et al. [8], the degree of DR was evaluated (DR score, 0-3) (Table 1). According to the system by Scheuer [9], 54 liver specimens from patients with viral hepatitis B and C infection were evaluated for necroinflammatory activity grading (score, 0-4) and for fibrosis staging (score, 0-4). Using the previously proposed system [10], 14 liver specimens from patients with NASH were evaluated for grading (score, 1-3) and for staging (score, 1-4). Using the previously published methods [11], membranous and/or cytoplasmic CK7+ hepatocytes with relatively abundant cytoplasm and centrally located vesicular nuclei were graded as follows (CK7+ hepatocyte score): 0, none; 1, <1% CK7+ hepatocytes; 2, 2-5% CK7+ hepatocytes; 3, 6-20% CK7+ hepatocytes; 4, 21-30% CK7+ hepatocytes; and 5, >30% CK7+ hepatocytes. The number of CK7+ hepatocytes in the periportal areas and peri- CV areas were also evaluated, divided by the number of portal areas and CVs, respectively, and "periportal CK7+ score" and "peri-CV CK7+ score" were calculated, respectively.

Score	Findings
0	Absent or rare single CK7+ cholangiocytes or ductular reaction
1	Rare single or focal ductular reaction or focal clustering of CK7+ cholangiocytes* in periportal areas
2	Continuous ductular reaction or clusters of CK7+ cholangiocytes* occupying <50% of portal areas
3	Continuous ductular reaction or clusters of CK7+ cholangiocytes* occupying >50% of portal areas
CK7: cytokeratin 7 *CK7+ cholangiocytes with and without ductular structures	

Table 1: Semi-quantitative assessment of ductular reaction

Using unpaired t-test and chi-square test, we examined the association of IIBD score with clinicopathologic variables in all cases, and the associations with grading and staging scores in viral hepatitis and NASH cases. Using Spearman's coefficient and Mann-Whitney U-test, we also investigated the relationship between DR score and CK7+ hepatocyte-related scores. Statistical significance was set at $P<0.05$.

Results

IIBDs usually showed tangential and infrequently longitudinal features (Figure 1). They were occasionally accompanied by dilated sinusoid or thin-wall vessels, but not by portal fibrous stroma or triad features composed of portal venule, hepatic arteriole, and bile ductule. Both pathologists identified IIBDs in 53 specimens (65.2%), and the interobserver variation is summarized in Table 2. Observed agreement was obtained in 66 of 81 cases (81.5%), and the kappa coefficient was 0.51, indicating moderate agreement. In 8 of 15 disagreement cases (53.3%), the discrepant number of IIBDs was 1 versus 0. The numbers of IIBDs counted by one and the other pathologist ranged from 1 to 18 (mean, 4.5) and from 1 to 13 (mean, 3.7). Their mean number of IIBDs ranged from 0 to 9.33 (mean, 2.11) and IIBD score ranged from 0 to 0.80 (mean, 0.28). Thirty-three liver specimens (40.7%) showed significant IIBD score (>0.28). In 54 specimens (66.7%), CK7+ hepatocytes were scattered in periportal areas, partly concomitant with CK7+ DR (Figure 2a) and/or peri-CV areas (Figure 2b). CK7+ hepatocyte score, periportal CK7+ score, and peri-CV CK7+ score ranged from 0 to 5 (mean, 1.53), from 0 to 1 (mean, 0.23), and from 0 to 1 (mean, 0.20), respectively.

	Pathologist 1			
Pathologist 2		IIBD+	IIBD-	Total
	IIBD+	53§	4*	57
	IIBD-	11†	13	24
	Total	64	17	81
Po (observed agreement)=(53 + 13)/81 = 0.815 Pe (expected agreement)=[(64/81) × (57/81)] + [(17/81)×(24/81)] Kappa=(Po-Pe)/(1-Pe)=0.51 IIBD: intraparenchymal isolated bile ductule. *The detected number of IIBDs by Pathologist 1 was "1" in 2 cases and "2" in 2 cases. †The detected number of IIBDs by Pathologist 2 was "1" in 6 cases, "2" in 4 cases, and "3" in 1 case. §The mean numbers of IIBDs detected by Pathologist 1 and Pathologist 2 were "4.5" (range, 1-18) and "3.7" (range, 1-13), respectively.				

Table 2: Interobserver variation regarding recognition of IIBD on the basis of H&E-stained histology

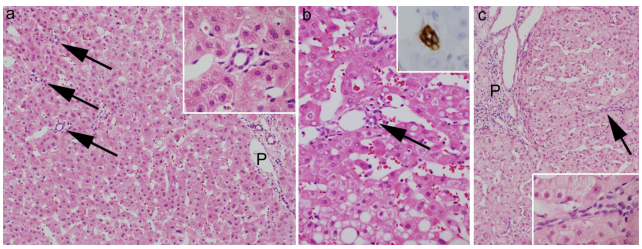

Figure 1: Intraparenchymal isolated bile ductules (IIBDs). (a) Scattered IIBDs (arrows) distant from a portal area (P), and a high-power view (inset) showing a small bile tubule composed of cuboidal cholangiocytes. (b) A tangential IIBD (arrow) with dilated sinusoidal spaces, and a high-power view (inset) showing its cytokeratin 7 expression. (c) A fibrously expanded portal area (P) and regenerative nodule-like lobule containing an IIBD (arrow), and a high-power view (inset) showing longitudinal features of IIBD

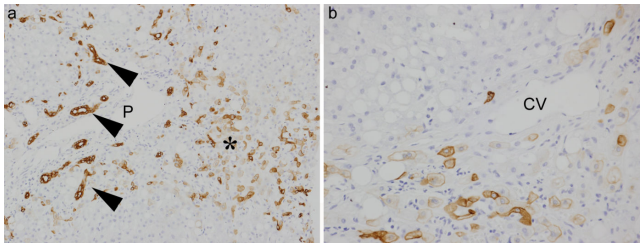

Figure 2: Cytokeratin 7 (CK7) expression of hepatocytes. (a) Periportal CK7+ hepatocytes (asterisk) and proliferated CK7+ bile ductules (arrowheads) in and around an expanded portal area (P), called ductular reaction. (b) Scattered CK7+ hepatocytes surrounding a centrolobular vein (CV)

Table 3 compares clinicopathologic variables between cases with and without significant IIBD score. Significant IIBD score was closely associated with CK7+ hepatocyte score (P=0.012), periportal CK7+ score (P=0.007), and peri-CV CK7+ score (P=0.032), but not with age, gender, all laboratory data examined, steatosis, or DR score. In 54 cases of viral hepatitis, significant IIBD score was not correlated with grading or staging score. However, in 14 cases of NASH, significant IIBD score was closely associated with higher grading score (P=0.043), but not with staging score.

	Cases of significant IIBD score (>0.28) (n=33)	Cases of non-significant IIBD score (0-0.28) (n=48)	P-value
Age (y), range (mean)	20-68 (44.0)	19-63 (44.2)	0.914*
Gender (male/female)	28/5	42/6	0.990†
Serum total bilirubin, range (mean)	0.35-2.19 (1.00)	0.41-3.51 (0.92)	0.499*
Serum AST, range (mean)	15-363 (68.8)	18-943 (95.9)	0.344*
Serum ALT, range (mean)	12-813 (92.6)	13-2035 (166.8)	0.214*
Serum ALP, range (mean)	103-893 (311.8)	144-1044 (274.9)	0.319*
Serum LDH, range (mean)	97-281 (189.3)	123-552 (192.0)	0.830*
Serum γ-GT, range (mean)	12-1888 (188.8)	16-419 (105.2)	0.141*
Steatosis score	0-3 (0.88)	0-3 (0.88)	0.988*
DR score (mean)	0-3 (1.67)	0-3 (1.48)	0.416*
CK7+ hepatocyte score (mean)	0-5 (2.03)	0-4 (1.19)	0.012*
Periportal CK7+ score (mean)	0-1.0 (0.34)	0-0.8 (0.15)	0.007*
Peri-CV CK7+ score (mean)	0-1.0 (0.29)	0-1.0 (0.15)	0.032*
Grading score in viral hepatitis cases (mean) [n=54]	0-3 (1.75) [n=20]	0-3 (1.50) [n=34]	0.808*
Staging score in viral hepatitis cases (mean) [n=54]	0-4 (1.75) [n=20]	0-3 (1.79) [n=34]	0.361*
Grading score in NASH cases (mean) [n=14]	2-3 (2.50) [n=4]	1-3 (1.60) [n=10]	0.043*
Staging score in NASH cases (mean [n=14]	2-3 (2.00) [n=4]	1-3 (2.10) [n=10]	0.827*

IIBD: intraparenchymal isolated bile ductule; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; CK7: cytokeratin 7; CV: centrilobular vein; DR: ductular reaction; LDH: lactate dehydrogenase; γ-GT: γ-glutamyltransferase; NASH, non-alcoholic steatohepatitis

*Unpaired t-test

†Chi-square test

Table 3: Clinicopathologic comparison of cases with and without significant IIBD score

DR score was closely correlated with CK7+ hepatocyte score, periportal CK7+ score, and peri-CV CK7+ score (Spearman's coefficient; all, P<0.001). When these data of hepatocytic CK7+ expression (CK7+ hepatocyte score, periportal CK7+ score, and peri-CV CK7+ score) were divided into 2 groups according to DR score (0-1 versus>1), there were significant differences between them (P=0.004, P=0.024 and P=0.031, respectively; Table 4).

	Cases of DR score, 0-1 (n=43)	**Cases of DR score, >1 (n=38)**	**P-value**
CK7+ hepatocyte score (mean)	0-3 (0.74)	0-5 (2.42)	0.004*
Periportal CK7+ score (mean)	0-0.8 (0.08)	0-1.0 (0.40)	0.024*
Peri-CV CK7+ score (mean)	0-0.57 (0.07)	0-1.0 (0.35)	0.031*

CK7: Cytokeratin 7; IIBD: Intraparenchymal Isolated Bile Ductule; Peri-CV: Peri-Centrilobular Vein; DR: Ductular Reaction

*Mann-Whitney U-test

Table 4: The comparison of periportal ductular reaction with hepatocytic CK7 expression

Discussion

In this study, on the basis of H&E-stained histology, 2 pathologists agreed in their identification of IIBDs in 65% of the needle biopsy specimens of the liver, denoting that they are not rare. The current kappa statistic indicated moderate agreement. However, in 53% of disagreement cases, one pathologist detected only 1 IIBD, while the other did not detect any. Only 1 IIBD would be hardly recognizable, and should be included in the same category as "0". Therefore, when the data were divided into 2 groups according to the number of IIBDs (0-1 versus>1), the kappa statistic was 0.60, suggesting a substantial agreement, despite no changes in observed agreement (81.5%; Table

5). These results indicate that histologic examination of H&E-stained liver biopsy specimens would be useful to detect IIBDs.

	Pathologist 1			
Pathologist 2		**The number of IIBDs, > 1**	**The number of IIBDs, 0-1**	**Total**
	The number of IIBDs, > 1	44	7	51
	The number of IIBDs, 0-1	8	22	30
	Total	52	29	81
Po (observed agreement)=(44 + 22)/81 = 0.815				
Pe (expected agreement)=[(52/81) × (51/81)] + [(29/81) × (30/81)]				
Kappa=(Po-Pe)/(1-Pe)=0.60				
IIBD: intraparenchymal isolated bile ductule				

Table 5: Revised interobserver variation regarding recognition of IIBDs on the basis of H&E-stained histology

The significance of IIBDs remains poorly understood, although Ryan et al. described IIBD as one type of chemotherapy-induced liver injury associated with hepatocytic apoptosis [6]. In the current study, significant IIBD score was not associated with increased serum level of AST, ALT, ALP, LDH, or γ-GT, and failed to reveal a close association with the severity of liver damage. An association with steatosis was not found, either. In both viral hepatitis and NASH cases, significant IIBD score was not associated with staging score, suggesting no relationship with liver fibrosis. However, in NASH cases, significant IIBD score was closely associated with higher grading score, although it was not in viral hepatitis cases. These findings suggest that the development of IIBDs is correlated with necroinflammatory severity in NASH cases, the pathogenesis of which might be somewhat different from that in viral hepatitis cases.

In normal liver, hepatocytes are usually negative for CK7 [12], but can aberrantly express CK7 in various conditions, possibly highlighting hepatic progenitor cell (HPC) activation [8,13]. CK7+ hepatocytes are frequently found in periportal areas, which are postulated to be conventional sites of HPC niches [14], but are also detectable in peri-CV areas [11]. In the current study, significant IIBD score was closely associated with all hepatocytic CK7+ indicators (CK7+ hepatocyte, periportal CK7+, and peri-CV CK7+ scores), suggesting the close relationship between the development of IIBDs and HPC activation.

A proliferation of bile ductules in and around portal areas, referred to as DR, occurs particularly in chronic cholestatic diseases [5], and we originally speculated that IIBDs may partly represent such DR. DR is also known to be relevant to aberrant hepatocytic CK7 expression [8,11,15]. In fact, the present study revealed a close relationship between DR score and all hepatocytic CK7+ indicators. However, interestingly, significant IIBD score was not statistically correlated with higher DR score. These results suggest that IIBDs would not be due to DR or to periportal niche-dependent HPC activation, which is similar to the aberrant CK7 expression of peri-CV hepatocytes. Moreover, IIBDs appeared to be distant from portal areas, but their anatomical association with the preexisting biliary tree was unclear in the current study. To elucidate these issues, further examinations are required.

Thus, IIBDs are recognizable in the routine examination of H&E-stained liver biopsy specimens, which are not rare. Significant IIBDs would be associated with hepatocytic CK7 expression or periportal niche-independent HPC activation. The development of IIBDs may be due in part to necro-inflammatory severity in NASH cases, but not in viral hepatitis cases.

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