Studia Universitatis "Vasile Goldiş", Seria Ştiinţele Vieţii
Vol. 21, issue 2, 2011, pp. 343-348

HEPATIC BILE DUCT LESIONS IN PATIENTS WITH CHRONIC HEPATITIS C; PREVALENCE AND SIGNIFICANCE

M. CORNIANU[1], A. DEMA[1], S. TĂBAN[1], D. LAZĂR[2], R. ŞIRLI[2], E. LAZĂR[1], A. FAUR[1], C. LĂZUREANU[1], I. MUNTEAN[3],R. POPESCU[3], R. CORNEA[1]
[1] Department of Pathology; [2] Gastroenterology Clinic;
[3] Department of Cellular Biology, U.M.F. "V. Babeş" Timişoara

ABSTRACT

Introduction: Hepatic bile duct lesions (BDL), lymphoid aggregates or lymphoid follicles (LA/LF) and hepatic steatosis (HS) are common histological features, often observed in patients with chronic hepatitis C (VHC). The pathogenesis and clinical significance of hepatic BDL remain unclear.

Material and method: We evaluated the prevalence and clinical significance of BDL on a group of 189 liver biopsies from patients with chronic viral hepatitis (125 patients with VHC and 64 with VHB), trying therewith to compare clinical, biochemical and morphologic data in patients with and without BDL.

Results: The mean age of patients with BDL was 50.5 years, slightly higher than the one of patients without BDL (48.61 years). Analyzing the frequency and pathological significance of BDL and portal lymphocyte infiltrate in patients with VHC we noted: a higher frequency of BDL in patients with VHC than in those with VHB (55/125; 44% vs.14/64; 21.8%); a high level of serum alanin-aminotransferase (ALT) (93.83 U/l), alkaline phosphatase (AP) (175.83 U/l) and gamma-glutamil-transpeptidase (γGTP) (99.76 U/l) in patients with BDL vs. 71.73 U/l, 146.76U/l and 71.24 U/l, respectively, in those without these kind of lesions; serum triglyceride (TG), cholinesterase (CHE) and total bilirubin (TB) levels were found to be significantly higher in patients without BDL. We did not observe a relationship between the sex of the patients and the presence of blood transfusions in their history, between the two groups of patients.

For the group of patients with VHC and BDL we observed a higher score of necroinflammation (9.87 vs. 7.38), portal inflammation (3.01 vs. 2.44) and fibrosis (1.65 vs. 0.97) and a higher frequency of LA/LF (49 vs. 36), as compared to patients without BDL. HS was found in 96 from the 125 (76.8%) patients with VHC, in 44 from 55 (80%) patients with BDL and in 52 from 70 (74.3%) of those without BDL.

Conclusion: The incidence of hepatic BDL is highly related to the severity of the histopathological lesions, being higher in patients with advanced liver disease and cholestasis. The implication and molecular role of hepatitis C virus in the pathogenesis of BDL requires further studies.

INTRODUCTION

Hepatic bile duct lesions (BDL), lymphoid aggregates or lymphoid follicles (LA/LF) and hepatic steatosis (HS) are characteristic histological features, often seen in patients with chronic hepatitis C (1,2). In these patients, the prevalence of BDL – defined as alterations of the biliary epithelium associated with lymphocyte infiltration, varies widely, between 15 and 91% (1,3).

These particular lesions of the bile ducts – named hepatitis-associated alterations of bile ducts or Poulsen-Christoffersen's bile duct lesions, were often reported in aggressive chronic viral hepatitis (1,2,4).

According to Goldin's criteria (1996) (5), in our study we judged as bile duct alterations, variable lesions of canalicular epithelium like stratification of epithelium, vacuolization, ballooning and cytoplasm eosinophilia, loss of nuclear polarity and pycnosis of nuclei.

MATERIAL AND METHOD

We evaluated the frequency of BDL on a group of 189 patients with viral chronic hepatitis, and also trying in the same time to compare clinical, biochemical and histopathological data in patients with and without BDL.

The patients that we studied were divided into two groups. The first group included 125 hepatic biopsy punctures (HBP) from patients with chronic hepatitis C (VHC) serologically confirmed (79 women and 46 men, with ages from 21 to 67 years old, mean age of 47.5 years). To compare the prevalence of hepatic BDL in patients with VHC, with the incidence of these lesions in other types of hepatitis, we evaluated the alteration of biliary canaliculi on a second group of patients (35 men and 29 women with ages between 17 and 68 years old, mean age 39.8 years) AgHBs-positive.

Hepatic sections stained with HE, tricrom Masson, van Gieson, PAS and orcein were evaluated using Knodell's Histological Activity Index (HAI). For both groups of patients (with and without BDL) we compared the histopathological lesions described by HAI: portal, periportal and lobular necroinflammation, the total score of necroinflammation and fibrosis; together with the conventional HAI histopathological evaluation, we also studied other characteristic features of VHC, like LA/LF and HS.

The lesions of biliary canaliculi, LA/LF and HS were quantified using the modified grading system described by Scheuer. We appreciated the severity of BDL using the sum of grades (0-2) (absent, mild or severe) following the criteria: infiltration with inflammatory cells, stratification and vacuolization of cytoplasm, degeneration of epithelial cells from interlobular ducts. LA/LF were graded: with 0- the absence of lymphoid infiltrate; with 1- weakly defined condensations of the lymphoid infiltrate; with 2- lymphoid aggregates or follicles well defined but without germinal centers, and with 3 was graded the presence of lymphoid follicles with germinal centers. Steatosis was graded in terms of the degree of severity as: absent (0), mild and moderate noted with 1 and severe noted with 2.

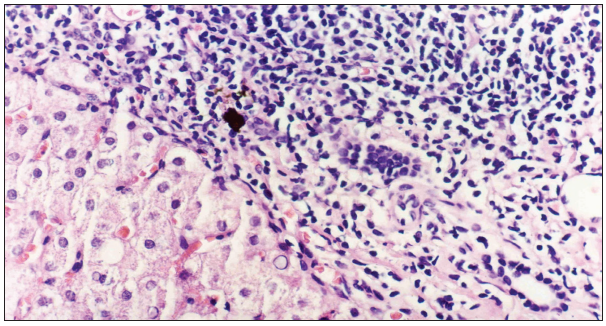


Fig. 1. Chronic hepatitis with C virus. Lesions of biliary canaliculi from portal space with stratification of epithelium and loss of nuclear polarity. HE x 400

From the 125 patients with VHC included in the study (46 men and 79 women, with ages between 21 and 67 years old, mean age of 47.5 years), 55(44%) (32 women and 23 men) were included in the group of patients with alterations of biliary canaliculi, with mild to severe lesions.

In only two cases, in some of the portal spaces, we observed estructions of canaliculi epithelium with loss of biliary canaliculi. The rest of 70 patients (56%) did not present lesions of bile ducts.

From the 64 patients with chronic hepatitis B (VHB), 14 (21.8%) (7 men and 7 women) presented mild to moderate lesions of biliary canaliculi. The frequency of BDL was significantly higher in patients with VHC

RESULTS

Bile duct lesions were defined according to Goldin's criteria as variable lesions of the canalicular epithelium. At the level of altered biliary canaliculi we observed degenerative lesions like: stratifications of bile duct epithelium with the loss of nuclear polarity (Fig. 1), vacuolization of duct epithelium (Fig. 2), ballooning or eosinophilia of the cytoplasm, nuclear pleomorphism with pycnosis of nuclei. In rare cases, in some of the portal spaces we noted the absence of bile ducts, an aspect that we interpreted as a destruction of bile canaliculi, and in two cases we identified aspects of metaplasia of the canaliculi epithelium.

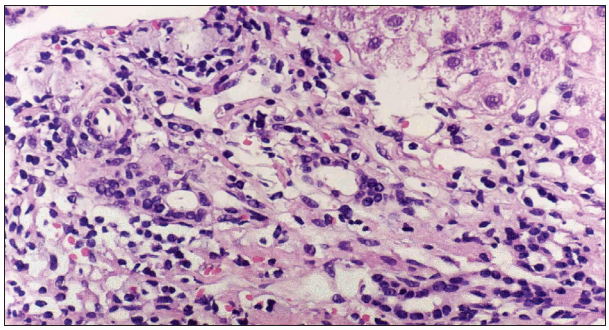


Fig. 2. Chronic hepatitis with C virus. Hyperplasia of biliary canalculi with stratification and vacuolization of duct epithelium. HE x 400.

than in those with VHB (55/125; 44% compared to 14/64; 21.8%).

Lymphocyte infiltration of bile duct epithelium, vacuolization and stratification of epithelium, as well as the presence of lymphoid aggregates in the vicinity of altered biliary canaliculi (Fig. 3), appreciated as hepatic BDL of severe degree, were identified in 17 cases; mild lymphocyte infiltration of biliary epithelium, noted in a few cases, was interpreted as a reduced degree of alteration of bile ducts from portal spaces.

From the 125 patients with VHC, 28 (22.4%) had blood transfusions, none of the patients had a history of renal dialysis or intravenous drug abuse.

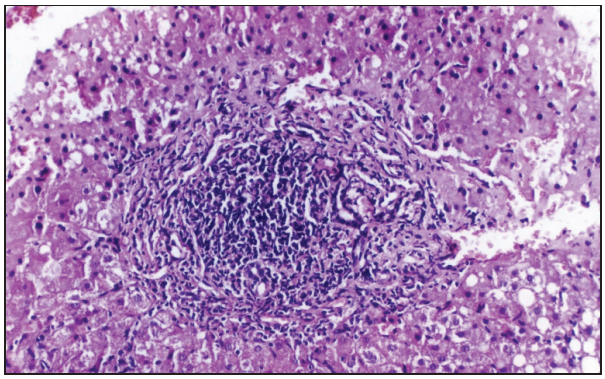


Fig. 3. Lymphoid aggregates situated in the vicinity of biliary canaliculi. HE x 200

In table 1 we present the comparative clinical and biochemical data of patients with VHC, with and without BDL.

We selected as variables sex and age, blood transfusions, biochemical data, the level of viremy and viral genotype (VHC 1b genotype being seen in the majority of cases), as well as hepatic histological modifications.

Tebel 1

Comparative clinical and biochemical data of patients with VHC, with and without BDL.

Clinical and biochemical data Patients with VHC	Patients with BDL (n=55)	Patients without BDL (n=70)
Sex (M/W)	23/32	23/47
Mean age (years)	50.5 (W: 48.25; M: 44.86)	48.61 (W: 48.97; M: 48.17)
Serum level of:		
ALT (U/l)	93.83	91.04
AST (U/l)	80.43	71.73
AP (U/l)	175.83	146.76
γ GTP (U/l)	99.76	71.24
TG (mg%)	94.19	103.5
CHE (U/l)	9909.9	10394.1
TB (mg/dl)	0.98	1.24
Viremy (KIU/ml)	836.5	709.33

ALT-alanin-aminotransferase; AST-aspartat-aminotransferase; AP-alcaline phosphatase; γGTP-gamma-glutamil-transpeptidase; TG-trigliceride; CHE-cholinesterase; TB-total bilirubin

Patients with lesions of biliary canaliculi had a significantly higher level of serum alanin-aminotransferase (ALT) and aspartat-aminotransferase (AST) than those without lesions of biliary canaliculi (ALT = 93.83 U/l vs. 71.73 U/l).

Patients with lesions of biliary canaliculi had a significantly higher level of serum alanin-aminotransferase (ALT) and aspartat-aminotransferase (AST) than those without lesions of biliary canaliculi (ALT = 93.83 U/l vs. 71.73 U/l). Between the two groups of patients we also noted significant differences of the serum level of alkaline phosphatase (AP) (175.83 U/l vs. 146.76 U/l) or gamma-glutamil-transpeptidase (γGTP) (99.76 U/l as compared to 71.24 U/l).

The mean age of patients with BDL was 50.5 years, slightly higher than the one of patients without BDL (48.61 years). But we did not observe noticeable differences as regard to the sex and the presence of blood transfusions in antecedents between the two groups of patients; the serum level of triglyceride (TG), cholinesterase (CHE) and total bilirubin (TB) was significantly higher in patients without BDL.

Viremy – determined in 24 cases, had a mean value of 836.5 KIU/ml for the patients with BDL, being much higher than the one of patients without these lesions (709.33 KIU/ml).

In table 2 we show the comparative hepatic histological features of patients with VHC, with and without hepatic BDL.

Patients with VHC and BDL had a significantly higher mean of periportal necroinflammation (4.96 as compared to 3.2), portal inflammation score (3.01 vs. 2.44), total necroinflammation (9.87 vs. 7.38), fibrosis score (1.65 as compared to 0.97), a higher frequency of lymphoid aggregates or follicles (49 vs. 36), than patients without this type of lesions.

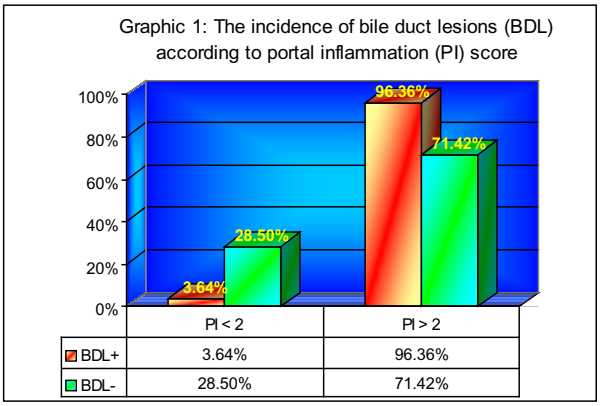
Table 2

Comparative hepatic histological features of patients with VHC, with and without hepatic BDL.

Histological features	Patients with BDL (n=55)	Patients without BDL (n=70)
Mean score for:		
· Periportal necroinflammatory activity	4.96	3.2
· Lobular necroinflammation	1.89	1.74
· Portal inflammation	3.01	2.44
· Total score of necroinflammation	9.87	7.38
· Fibrosis	1.65	0.97
Lymphoid aggregates or follicles		
· Score 0; 1; 2; 3	6; 16; 32; 1	34; 21; 13; 2
· Score 0, 1 : 2, 3	22: 33	55: 15
Steatosis		
· Score 0: 1: 2	11: 33: 11	18: 41: 11

53 (96.36%) of the 55 patients with lesions of biliary canaliculi had a score of portal inflammation ≥ 2, significantly higher than 50 (71.42%) of the 70 patients without lesions of biliary canaliculi (Graphic 1).

Graphic 1: The incidence of bile duct lesions (BDL) according to portal inflammation (PI) score

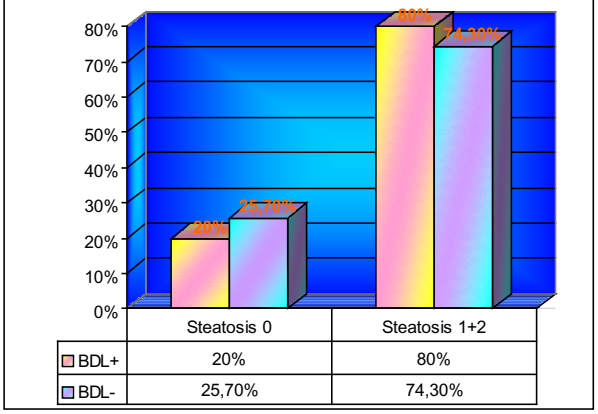


	PI < 2	PI > 2
BDL+	3.64%	96.36%
BDL-	28.50%	71.42%

Well-defined lymphoid aggregates and lymphoid follicles were observed in 49 cases with BDL, in one case the presence of lymphoid follicles being associated with formation of germinal centers. 33 (60%) of the 55 patients with lesions of biliary canaliculi had a LA/LF score ≥ 2, more than the 15 patients without lesions of biliary canaliculi.

Hepatocyte steatosis was found in 96 of the 125 (76.8%) patients with VHC, in 44 of the 55 (80%) patients with lesions of biliary canaliculi and in 52 of the 70 (74.3%) patients without BDL (Graphic 2). 11 of the patients with BDL had a marked steatosis (2+), which affected more than 1/3 of hepatocytes.

Graphic 2: The incidence of hepatic steatosis in patients with and without BDL



	Steatosis 0	Steatosis 1+2
BDL+	20%	80%
BDL-	25,70%	74,30%

DISCUSSION

On a group of 189 HBP from patients with VHB (64 cases) and VHC (125 cases) we evaluated the frequency of BDL, comparing clinical, biochemical data and hepatic histologic modifications (portal, periportal and lobular necroinflammation; fibrosis; LA/LF and steatosis) in patients with and without BDL.

55 (44%) from the 125 patients with VHC presented lesions of biliary canaliculi, this incidence being situated within the limits reported by previous studies, which noticed a frequency between 15 and 91% (1,3).

Hwang et al. (2001) (6) reported the presence of BDL in 83 (71%) of 117 patients with serologically confirmed VHC, while Wong et al. (1996) (3) described affected bile ducts in 30 (15%) of the 200 studied cases, in tight connection with the presence of lymphoid aggregates. In other studies – Haruna et al. (13) noted the presence of bile duct alterations in 86 (55%) of the 112 patients, in 11 cases observing severe necrosis of canaliculi epithelium and their destruction.

Although lymphocyte infiltration of bile ducts associated with degeneration of epithelium is relatively common in VHC, a destructive cholangiopathy is extremely rare (5). While Bach et al. (1992) (2) reported hepatic BDL and disappearance of bile ducts in 91% of cases, Scheuer (1992) (1) described significant lesions in 6% of cases and less severe lesions in 17% of patients. Similar to the results reported by Hwang, in our study, the prevalence of BDL was significantly higher in patients with VHC (55 of 125; 44%) than in AgHBs-positive patients (14 of 64; 21.8%).

The relationship between hepatic BDL and other histologic features like LA/LF, HS and hepatic necroinflammatory activity has a clinical interest. Preliminary studies noted the high frequency of these hepatic lesions in patients with VHC, in association with severe necroinflammation and in tight connection with the presence of portal lymphoid aggregates or lymphoid follicles (1,7,8,9). In our study, the 55 patients with VHC and BDL presented a mean of periportal necroinflammation (4.96 vs. 3.2), portal inflammation (3.01 as compared to 2.44), fibrosis (1.65 vs. 0.97) and total necroinflammation (9.87 as compared to 7.38) significantly higher; we also observed a high frequency of BDL in association with the presence of LA/LF in the portal space.

Hwang sustains that patients with hepatic BDL have a significantly higher score for LA/LF, portal inflammation, necroinflammatory activity and hepatic fibrosis, as compared to patients without this kind of lesions (6). The multivariate logistic analysis of these cases shows portal inflammatory activity and the presence of LA/LF, as significant predictors of BDL; these observations delineate the hypothesis that the presence of hepatic biliary canaliculi alterations is closely related to the severity of portal inflammation and the presence of portal LA/LF (10,11).

The clinical significance of BDL presence in patients with VHC is not entirely clarified. Hwang does not observe significant differences in what concerns clinical (sex, mean age, blood transfusions) and hepatic biochemical data, except globulinemia, between patients with and without BDL. A significantly higher mean value of globulinemia in the group of patients with BDL can

signify a more severe hepatic fibrosis or the presence of infection with VHC 1b genotype – both aspects suggesting a longer continuance of infection (6).

Our results confirm, in part, some of these studies, showing: a higher level of serum ALT and AST in patients with VHC and BDL (ALT 93.83 U/l; AST 80.43 U/l) than those without this type of lesions (ALT 91.04 U/l; AST 71.73 U/l); an association between the presence of BDL and the high level of AP (175.83 U/l vs. 146.76 U/l) or γGTP (99.76 U/l vs. 71.73 U/l).

The theory stating that chronic hepatitis with C virus is considered to be caused by the immune response of the host was sustained by studies that show the predominance of lymphocyte infiltrate in the liver infected with VHC and the lack of correspondence between the magnitude of VHC-viral replication and the severity of histopathological lesions.

Some studies sustain the implication of HLA DR in the development of chronic hepatitis VHC, suggesting that the lesions of biliary canaliculi and the portal lymphocyte infiltrate are caused by a host immune reaction, regulated by HLA DR (13).

Gerber et al. (7) consider that BDL are characteristic to VHC infection, showing that 90% of American and 55% of Japanese patients with chronic VHC infection had BDL, and these differences could be linked to the different HLA immune background of the two populations.

The pathogenesis and clinical significance of hepatic BDL in patients with chronic hepatitis C were not as yet clarified. Recent studies affirm that this lesions mime the non-suppurative destructive cholangitis described in patients with primitive biliary cirrhosis (4,8). Although the patients with VHC present frequently immune manifestations such as positive serum antibodies (anti-nuclear antibodies – ANA, smooth muscle antibodies – SMA etc), crioglobulinemia and sialadenitis (9,12), the relationship between BDL and these immune manifestations was not yet clarified.

The results of Hwang's study did not show significant differences in the manifestation of some immune bad regulations including the presence of crioglobulinemia and of serum antibodies (ANA, SMA, AMA) between patients with and without biliary canaliculi lesions. These observations demonstrate that immune mechanisms involved in the development of hepatic BDL in hepatitis C are not associated with serum crioglobulins and antibodies (6).

Using modified PCR and immunohistochemistry (IHC), the existence of VHC in bile and epithelial cells of biliary canaliculi was demonstrated. The detection of VHC genotype in biliary epithelium by HIS was strongly correlated with bile duct lesions. All these observations suggest that BDL can be caused by an immune reaction of the host against the epithelial cells of biliary canaliculi infected with VHC.

In conclusion, in patients with chronic hepatitis C, bile duct lesions and portal lymphocyte infiltrate seem to be caused by the immune reaction of the host, regulated by HLA DR (13).

REFERENCES

Scheuer PJ, Ashrafzadeh P, Sherlock S, Brown D, Dusheiko GM. The pathology of hepatitis C. Hepatology 1992; 15:567-571.

Bach N, Thung SN, Schaffner F. The histological features of chronic hepatitis C and autoimmune chronic hepatitis: a comparative analysis. Hepatology 1992; 15:572-577.

Wong VS, Wight DGD, Palmer C.R, Alexander GJM. Fibrosis and other histological features in chronic hepatitis C virus infection: a statistical model. Journal of Clinical Pathology 1996; Vol. 49, No. 6: 465-469.

Ludwig J, Czaja AJ, Dickson ER. Manifestations of nonsuppurative cholangitis in chronic hepatobiliary diseases: morphologic spectrum, clinical correlations and terminology. Liver 1984; 4: 105-10.

Goldin RD, Patel NK, Thomas HC. Hepatitis C and bile duct loss. J Clin Pathol, 1996; 49:836-838.

Hwang SJ, Luo JC, Chu CW et al. Clinical, virological, and pathological significance of hepatic bile duct injuries in Chinese patients with chronic hepatitis C. Journal of Gastroenterology, 2001; 36:392-398.

Gerber MA, Krawczynski K, Alter MJ, Sampliner RE. Margolis HS, and the sentinel counties chronic non-A, non-B hepatitis study team. Histopathology of community acquired chronic hepatitis C. Mod Pathol, 1992; 5:483-486.

Kaji K, Nakamuma Y, Sasaki M et al. Hepatitic bile duct injuries in chronic hepatitis C: histopathological and immunohistochemical studies. Mod Pathol, 1994; 7:937-945.

Luo JC, Hwang SJ, Lai CR et al. Clinical significance of portal lymphoid aggretates/follicles in Chinese patients with chronic hepatitis C. Am J Gastroenterology, 1999; 94:1006-1011.

Danque POV, Bach N, Schaffner F, Gerber MA, Thung SN. HLA-DR expression in bile duct damage in chronic hepatitis C. Mod Pathol 1993; 6: 327-32.

Hino K, Okuda M, Konishi T et al. Analysis of lymphoid follicles in liver of patients with chronic hepatitis C. Liver, 1992; 12:387-91.

Pawlotsky JM, et al. immunological disorders in C virus chronic active hepatitis: a prospective case-control study. Hepatology, 1994; 19:841-848.

Haruna Y, Miyamoto T, Yasunami R, Kanda T, Fushimi H. Human leucocyte antigen DRN1 1302 protects against bile Duch damage and portal lymphocyte infiltration in patients with chronic hepatitis C. Journal of Hepatology 2000; 32: 837-42