Hepatitis B Infection in Patients With Acute Liver Failure in the United States

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Occult hepatitis B virus (HBV) infection has been reported in 30% to 50% of patients with acute liver failure (ALF) in small case series. The aim of this study was to determine the prevalence of occult HBV infection in a large series of ALF patients in the United States and the prevalence of precore and core promoter variants in patients with ALF caused by hepatitis B. Sera from patients in the US ALF study and liver, when available, were tested using nested polymerase chain reaction (PCR) with primers in the HBV S and precore regions. PCR-positive samples were sequenced. Sera and/or liver from 139 patients (39 males, 100 females; mean age, 42 years) enrolled between January 1998 and December 1999 were studied. Twelve patients were diagnosed with hepatitis B, 1 with hepatitis B+C+D coinfection, and 22 had indeterminate etiology. HBV DNA was detected in the sera of 9 (6%) patients; all 9 had ALF caused by hepatitis B. HBV genotypes A, B, C, and D were present in 4, 3, 1, and 1 patients, respectively. Seven of these 9 patients had precore and/or core promoter variants. Liver from 19 patients were examined. HBV DNA was detected in the liver of 3 patients with ALF caused by hepatitis B, but in none of the remaining 16 patients with non-B ALF. Contrary to earlier reports, occult HBV infection was not present in this large series of ALF patients in the United States. HBV precore and/or core promoter variants were common among US patients with ALF caused by hepatitis B. (HEPATOLOGY 2001; 33:972-976.)

There are approximately 2,000 cases of acute liver failure (ALF) in the United States each year.[1] A retrospective analysis of 295 cases found that acetaminophen overdose was the most common etiology of ALF in the US.[2] In this series, 10% had hepatitis B and 15% had no identifiable cause.

Several studies reported that some patients with ALF, despite testing negative for hepatitis B surface antigen (HBsAg) and hepatitis B core antibody immunoglobulin M (anti-HBc IgM), had detectable hepatitis B virus (HBV) DNA in the serum or liver using polymerase chain reaction (PCR) assays.[3-8] In these patients, the etiology of ALF may be related to occult hepatitis B infection. The prevalence of occult hepatitis B infection in patients with non-A, non-B ALF has been reported to vary from a low of 0% to 4% in Europe[9-12] to 50% in Japan.[3-5] Available data on the prevalence of occult hepatitis B infection among patients with ALF in the US are conflicting and based on very few studies in a small number of patients. The prevalence of occult hepatitis B infection in patients with ALF was reported to be 30% to 50% in 3 studies that involved a total of 31 patients,[6-8] but absent in 2 other studies that involved a total of 23 patients.[13,14] Studies that examined both sera and liver tissues invariably reported higher rates of HBV detection in the liver.[3,4,7] Liver tissues were tested in 4 of the 5 US studies cited.

Among patients with ALF secondary to hepatitis B infection (ALF-B), an association between mutations in the precore and core promoter region of the HBV and a fulminant course of the disease has been reported. HBV precore stop codon variants had been detected in 88% to 100% of patients with ALF in Japan,[15,16] 83% in Israel,[17] 36% in Taiwan,[18] 10% in France,[19] and only 5% in the US.[20] A subsequent study found that only 7% of patients with ALF in the US had predominant precore variants, but 53% had a mixture of precore variants and wild-type HBV.[21] The low frequency of precore variants in the US is thought to be related to the predominance of HBV genotype A, which precludes the selection of the G-to-A change at nucleotide 1896.[22] HBV core promoter variants had been found in 68% to 72% of patients with ALF-B in Japan,[23,24] and in 30% to 78% in Germany.[25,26] The data on prevalence of core promoter variants in patients with ALF in the US are limited. A single US study reported that 10% of patients with ALF-B had core promoter variants.[27]

The aims of this study were to determine the prevalence of occult hepatitis B infection in a large cohort of patients with ALF in the US and to determine the prevalence of HBV precore and core promoter variants among US patients with ALF secondary to hepatitis B.

PATIENTS AND METHODS

Materials

Serum samples were prospectively collected from patients presenting with ALF of any etiology to the 14 academic centers participating in the US ALF Study Group (see Appendix). All but 1 are liver transplantation centers. ALF was defined as the presence of coagulopathy (prothrombin time >15 seconds or international normalized ratio >1.5) and the onset of hepatic encephalopathy within 8 weeks of the first symptoms or jaundice in an individual without underlying liver disease.[28] Serum samples were collected on the day of presen-

Abbreviations: ALF, acute liver failure; HBsAg, hepatitis B surface antigen; anti-HBc IgM, hepatitis B core antibody immunoglobulin M; HBV, hepatitis B virus; PCR, polymerase chain reaction; HBeAg, hepatitis B e antigen.

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tation and daily for 7 days. Samples at presentation and, when available, a second specimen collected between days 2 to 7 were tested for HBV DNA. All the serum samples were stored at −80°C before testing. Collection of liver tissues was not included in the ALF study protocol. Liver specimens, when available at autopsy or transplantation, were retrieved as formalin-fixed, unstained sections and tested for HBV DNA. Both the serum samples and liver tissues were tested under code in Dr. Lok's laboratory at the University of Michigan Medical Center. Each center provided detailed demographic, clinical, laboratory, and outcome information for all patients enrolled. Etiologic categories were defined by each study center, based on accepted diagnostic criteria that were circulated to all investigators. The etiology was attributed to hepatitis B when HBsAg and/or anti-HBc IgM were positive. The diagnosis of indeterminate etiology was made when extensive history, clinical evaluation, and laboratory tests failed to reveal a diagnosis. All patients' data were collected at the individual sites and sent to the central site at University of Texas Southwestern Medical Center (UTSW). Data were then entered into an Access (Microsoft) database by staff of the Academic Computing Department at UTSW. This study was approved by the Institutional Review Boards of all centers involved. Informed consent was obtained from the next of kin, because patients had altered mentation as an entry criterion. Information on the demographic data, clinical etiology, serologic markers of hepatitis A, B, and C, and outcome of the patients were released by the ALF study coordinating center after the HBV-DNA test results by PCR were reported.

PCR Assays for HBV DNA

Serum Samples. Serum samples were tested for HBV DNA by nested PCR assay using primers to amplify 2 different regions of the HBV genome: the precore/core region and the surface region as described previously.[29,30] All necessary precautions were taken to prevent cross-contamination. Negative controls using sterile water instead of DNA extract and positive controls using cloned plasmids containing the whole HBV-DNA genome were run with every batch of serum samples. The sensitivity limit of our PCR assay is 250 to 500 copies of HBV DNA per milliliter.[29] Samples with no detectable HBV DNA were retested using twice as much first-round PCR product.

Liver Tissues. Liver specimens from 19 patients were available for analysis. DNA was extracted using the Ex-Wax DNA Extraction Kit (Intergen Co., Purchase, NY) according to the manufacturer's instructions. Explanted liver tissues from 2 patients with hepatitis B were used as positive controls for DNA extraction and subsequent PCR. Explanted liver tissues from 2 patients with primary biliary cirrhosis and no serologic marker of HBV infection were used as negative controls. Because of the limited availability of liver tissue (1-3 sections) from the study patients, PCR was performed using one set of primers from the precore/core promoter region only. We were able to detect HBV DNA from the positive controls with as little as 1/10 of the DNA extract from one section (5 μm thick) of liver tissue. To optimize the yield in the study patients, we used 3 to 10 times as much DNA extract and 5 to 10 times as much first-round PCR product compared with the minimal amount required for the detection of HBV DNA in our positive controls.

Sequencing of HBV Genome

Amplified PCR products from serum samples or liver tissues were purified by Qiaquick spin columns (Qiagen Inc., Chatworth, CA) and analyzed by direct sequencing using the standard protocol for the Applied Biosystems DNA sequencer 373A (Perkin-Elmer Corp., Foster City, CA). Primers SS2 (5′-3′ nucleotide 61-75) and SAS2 (5′-3′ nucleotide 991-970) were used for direct sequencing of the S gene, and P1 (5′-3′ nucleotide 1604-1623) and P2 (5′-3′ nucleotide 2076-2060) were used for the precore/core promoter region.[29,30] The DNA sequences of the S gene were compared with published sequences in Genbank to determine the HBV genotypes.

RESULTS

Demographics of the Patients Studied. Between January 1998 and December 1999, 179 patients were enrolled in the US ALF study. Forty patients did not have stored serum sample or liver tissue and were excluded from this study. Of the 139 patients (78% of the entire series) studied, 39 were males and 100 were females. The mean (±SD) age was 42 ± 14 years. There were 101 whites, 20 Hispanics, 9 African Americans, 5 Native Americans, 3 Asians, and 1 patient of unknown race. Paired serum samples were available in 108 patients, including 7 patients with ALF-B and 101 patients with non-B ALF.

Clinical Etiology of ALF. The most common clinical etiology in our cohort of ALF patients was acetaminophen overdose (34%) (Table 1). Twenty-two patients (16%) appeared to have no clear etiology. ALF-B was diagnosed in 12 patients (9%). One patient had triple viral infection with hepatitis B, C, and D.

HBV-DNA Detection in Serum Samples. HBV DNA was detected using PCR assay in serum samples from 9 (6%) patients. All 9 patients had a clinical diagnosis of ALF-B and detectable HBV DNA using both sets of primers. Five of these 9 patients had follow-up samples. HBV DNA remained detectable in 2 (40%) patients, 2 to 5 days after presentation. HBV DNA was not detected in the remaining 3 patients with ALF-B nor in the patient with triple viral infection. Six patients with ALF-B were tested for HBV DNA by non-PCR based assays at the participating centers: none tested positive. Four (67%) of these 6 patients had detectable HBV DNA by our PCR assay.

HBV DNA could not be detected in any of the 126 patients with non-B ALF, including 22 patients with indeterminate etiology. Anti-HBc IgG was tested in 98 patients with non-B ALF: only 7 (7%) tested positive.

HBV-DNA Detection in Liver Tissue. Of the 19 patients with liver specimens, 8 patients had indeterminate etiology, and 3 had ALF-B (Table 2). HBV DNA was detected in the liver tissues from all 3 patients with ALF-B, but in none of the 16 patients with non-B ALF. Fifteen of the 16 patients with non-B ALF were tested for anti-HBc IgG: none tested positive. Serum

TABLE 1. Clinical Etiology of ALF and Prevalence of HBV Markers

Clinical Etiology	N (%)	HBV DNA + by PCR in Serum	HBV DNA + by PCR in Liver Tissue	$HBsAg^+$	Anti-HBc IgM^+	Anti-HBc Total +
Acetaminophen overdose	47 (34)	0	0/2	0/42	0/15	5/35
Indeterminate	22 (16)	0	0/8	0/21	0/12	0/17
Drug-induced	21 (15)	0	0/3	0/20	0/6	1/16
Hepatitis A	8 (6)	0	0/0	0/8	0/3	1/5
Hepatitis B	12 (9)	9	3/3	10/12	11/12	7/8
Hep B + C + D	1 (0.7)	0	0/0	1/1	0/1	0/1
Miscellaneous	28 (20)	0	0/3	0/25	0/13	0/24
Total	139	9	3/19	11/129	9/60	14/106

TABLE 2. Characteristics of the 3 Patients With Intrahepatic HBV DNA

Patient No.	HBV DNA by Non-PCR Assay	HBV DNA by PCR in Liver Tissue	HBV DNA by PCR in Serum	HBsAg	Anti-HBc IgM	Anti-HBc Total	HBeAg
1	N	P	P	P	P	P	P
2	N	P	P	P	P	P	N
3		P	P	P	N	P	N

samples were not available in 2 patients who had stored liver tissue. The results on the liver and serum specimens were concordant in the remaining 17 patients.

HBV Genotyping and Sequencing. Of the 9 patients with ALF-B, 4 were infected with HBV genotype A, 3 with genotype D, 1 with genotype B, and 1 with genotype C.

Analysis of the precore region revealed mutations in 6 patients (Table 3). Four had the commonly described precore stop-codon mutation, eW28X/G_{1896}A (TGG to TAG). One patient also had a precore stop-codon mutation at position 28, but the G-to-A change involved nucleotide 1897, eW28X/G_{1897}A (TGG to TGA). The remaining patient had a precore start-codon mutation, G_{1817}T (ATG to ATT). All 6 patients were hepatitis B e antigen (HBeAg)-negative. All patients except the one with the eW28X/G_{1897}A (TGG to TGA) mutation were infected with non-A HBV genotypes.

Mutations in the core promoter region were seen in 3 patients (Table 3). Two patients had the dual mutations A_{1762}T and G_{1764}A, and 1 patient had the G_{1764}A mutation only. The latter patient had 2 other mutations, C_{1766}T and T_{1768}A, in the core promoter region. Two patients had concomitant mutations in the precore region. The third patient who had mutation in the core promoter region only was HBeAg-positive. Overall, 7 of the 9 (78%) patients with fulminant hepatitis B had mutations in the precore or core promoter region.

Analysis of the HBV S gene did not reveal any consistent mutations. Most of the changes were at sites that are polymorphic across the HBV genotypes. None of the remaining changes were detected in more than 1 of the 9 patients studied.

DISCUSSION

In our study, which represents the largest series of consecutive patients with ALF in the United States,[6-8,13,14] we could not find a single case of occult hepatitis B infection. Several studies have reported that using PCR assays, HBV DNA may be detected in the serum of individuals who have recovered from prior hepatitis B infection.[31] The low rate of occult hepatitis B infection observed in our study and in a similar study of Sallie et al.,[10] compared with reports from Japan, may be related to a lower prevalence of past HBV infection in the United States and England. In support of this hypothesis, only 7% of the patients in our study with non-B ALF tested positive for anti-HBc IgG. While the low rate of detection of occult hepatitis B infection might be related to the relative insensitivity of our PCR assays, our standard PCR assay has a detection limit of 250 to 500 copies of HBV DNA per milliliter of serum or 25 to 50 copies of HBV DNA per assay. This is comparable with commercially available PCR assays. In addition, all the samples that tested negative were retested using 2-fold higher concentrations of DNA. Therefore, it is unlikely that low sensitivity of the PCR assay accounts for the negative results.

Mutations in the HBV genome may be present, especially in patients with occult hepatitis B infection. To ensure optimal detection of HBV DNA, we performed nested PCR using primers from 2 regions of the HBV genome. All the samples that tested positive had concordant results using both sets of primers. In this study, HBV DNA was detected in the sera of 9 (75%) and in the liver tissues of all 3 patients with ALF-B. These rates of detection are similar to other published reports.[21]

Patients with ALF-B have rapid viral clearance.[32] Failure to detect HBV DNA in the sera of 3 patients with ALF-B may be related to rapid viral clearance or late referral of patients to the participating sites. Of the 7 ALF-B patients with follow-up samples, 2 had undetectable HBV DNA at presentation. In the remaining 5 patients with HBV DNA in serum at presentation, HBV DNA became undetectable in 3 (60%) patients after 3 to 6 days, illustrating the rapidity of viral clearance in patients with ALF-B. Several studies reported that coinfection with hepatitis C or D virus may interfere with HBV replication.[33] Viral interference may account for the lack of detectable HBV DNA in the patient with triple hepatitis virus infection.

Previous studies on patients with occult hepatitis B infection found that HBV DNA is more often detected in liver tissues than in sera.[3,4,7] Unfortunately, liver tissues are rarely available in patients with ALF. In this study, liver specimens

TABLE 3. HBV Genotypes, Precore, and Core Promoter Changes in Patients With Fulminant Hepatitis B

Patient No.	Race	Sex	Age	HBeAg	HBV Genotype	Core Promoter		Precore
						Nt_{1762}	Nt_{1764}	Nt_{1896}
1	White	M	54	Neg	D	A	G	A
2	White	M	63	Neg	C	A	G	A
3	Black	M	50	Pos	A	A	G	G
4	White	F	17	Neg	D	A	G	G_{1817}T
5	Black	F	43	Pos	A	T	A	G
6	Asian	F	62	Neg	B	A	G	A
7	White	M	58	Pos	A	A	G	G
8	White	M	62	Neg	D	T	A	A
9	White	F	30	Neg	A	A	A	G_{1897}A

were available for analysis in 19 (14%) patients only. The low percent of patients with liver specimens was related to the fact that collection of liver tissues was not included in the ALF study protocol. HBV DNA was detected in the liver tissues of all 3 patients with ALF-B, but in none of 16 patients with non-B ALF. These 3 patients underwent transplantation on days 2, 5, and 7 after presentation. Follow-up serum was not available in the first patient, but in the latter 2, sera collected on days 5 and 6 of illness, respectively, tested negative for HBV DNA by PCR. The lack of liver tissues for analysis in 86% of the study patients may have resulted in an underestimation of the prevalence of occult hepatitis B infection. Despite these limitations, our sample size of 19 liver specimens is larger than other published reports.[6,7,8,12] Thus, we believe that occult hepatitis B infection is extremely rare among patients with ALF in the US.

Precore stop-codon variants had been previously reported to be rare in the US, because the predominant HBV genotype A precludes the development of the $G_{1896}A$ mutation. However, we found that 6 (67%) of 9 patients with ALF-B had precore variants. Four of these 6 patients had the classical $G_{1896}A$ change. The high prevalence of precore stop-codon variant among our patients may be related to the diversity of HBV genotypes, with 5 (56%) patients infected with genotypes B, C, and D, which permit the selection of the $G_{1896}A$ mutation. The diversity of HBV genotypes and a higher prevalence of precore stop-codon variants in our study compared with previous US studies[20,21] may be related to the fact that our study recruited patients from 14 centers across the United States. These "differences" may also be related to the small sample size in all studies performed to date, including our study. Core promoter variants were also reported to be rare in ALF patients in the United States.[27] Precore or core promoter variants were found in 78% of our patients. Mutations in the core promoter region involving $C_{1766}T$ and $T_{1768}A$ had been reported to enhance HBV replication.[34] These mutations were seen in 1 of our patients. Because of the small number of patients with ALF-B and the lack of controls with self-limited acute hepatitis B, we cannot determine if the precore and core promoter mutations are more prevalent in patients with a fulminant course.

In summary, based on our study of a large cohort of ALF patients, occult HBV infection is extremely rare in patients presenting with ALF in the United States. In fact, we did not detect a single case of occult hepatitis B infection in this study. Among patients with ALF-B, mutations in the precore and core promoter region were common. However, the relation between these mutations and the course of the hepatitis is not clear, because precore and core promoter variants have been found in inactive carriers as well as in patients with chronic liver disease.

APPENDIX

US ALF Study Group

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