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Viral Superinfection in Previously Unrecognized Chronic Carriers of Hepatitis B Virus with Superimposed Acute Fulminant versus Nonfulminant Hepatitis

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The role of viral superinfection in hepatitis B surface antigen carriers with superimposed fulminant ($n = 60$) versus nonfulminant ($n = 90$) acute hepatitis was studied. The frequency of hepatitis A virus (HAV) (0 versus 2.2%), HCV (18.3 versus 21.1%), HDV (15.0 versus 7.8%), and HEV (1.7 versus 4.4%) infection showed no significant difference, while simultaneous HCV and HDV infection was significantly more prevalent in the former (8.3 versus 0%). Only 3.6% of fulminant cases and 3.3% of nonfulminant controls were HGV RNA positive.

It has long been recognized that a substantial proportion of hepatitis B surface antigen (HBsAg)-positive patients with fulminant hepatitis in previous studies were negative for immunoglobulin M (IgM) antibody hepatitis B core antigen (IgM anti-HBc) (1, 3, 19, 21, 26, 28). These findings suggested that many HBsAg-positive patients with fulminant hepatitis were indeed previously unrecognized HBsAg carriers with acute exacerbation of chronic hepatitis B virus (HBV) infection or viral superinfection (2, 4, 10), though the possibility of acute HBV infection with poor IgM anti-HBc response due to mutation of HBV core gene cannot be completely excluded. Earlier studies revealed that 60 to 80% of HBsAg carriers with fulminant hepatitis had serological evidence of HDV infection (9, 26). Since the availability of HCV testing, serological evidence of HCV infection also has been detected frequently in these cases in several small reported series of studies (3, 7, 30). With the advent of serological and virological identification techniques, it is time to revisit the prevalence and significance of the hepatotropic virus as well as the newly discovered HGV superinfection in HBsAg carriers with superimposed fulminant versus nonfulminant acute hepatitis.

Patients. Sixty consecutive HBsAg-positive but IgM anti-HBc-negative patients, as assayed by radioimmunoassay (Abbott Laboratories), referred for fulminant hepatitis to our hospital in a 6-year period from 1990 to 1995, were studied. Forty-five were men and 15 were women; ages were 18 to 78 years (median age, 40 years). All received only supportive medical treatment, and 43 patients (88.3%) died shortly after hospitalization. The controls consisted of 90 age and sex-matched HBsAg-positive but IgM anti-HBc-negative patients with icteric nonfulminant acute hepatitis who were admitted to our hospital during the same period. Sixty-seven were men and 23 were women; ages were 18 to 84 years (median age, 40 years). All study patients and controls were previously healthy subjects without preexisting liver disease. They were first recognized as chronic HBsAg carriers while presenting these episodes of acute hepatitis. The onset and the likely mode of transmission of HBV infection in these cases were unknown, but chronic HBV infection in Taiwan is usually acquired perinatally or in early childhood (27). All patients denied homosexual activity or intravenous drug abuse. Drugs and alcohol were excluded as likely etiologic agents.

Serological tests. Serodiagnosis of acute non-B hepatotropic virus infection in HBsAg carriers was done by using commercial kits. The serological tests, assay name, and diagnostic criteria for acute HAV, HCV, HDV, and HEV superinfection are summarized in Table 1. Among patients who had no evidence of HAV, HCV, HDV, and HEV superinfection, acute reactivation of the underlying chronic HBV infection was suspected if serum HBV DNA was positive by spot hybridization (5), though the presence of HBV DNA in itself was not diagnostic of this event, and patients were presumed to have acute hepatitis of unidentified cause if serum HBV DNA was negative by spot hybridization, but the possibility of acute reactivation of chronic HBV infection with early clearance of viremia could not be completely excluded (15). The presence of serum HGV RNA, as detected by PCR with specific primers (5′-GAGAT TCCTTTTTATGGGCATGG-3′ and 5′-CACCAGGTCTCC GTCTTTGAT-3′) which were designed in accordance with the published consensus NS3 region of HGV (13), in the study cases and controls was then correlated with the established etiology of acute hepatitis.

Results and discussion. The results of serological tests for acute fulminant versus nonfulminant hepatitis superimposed upon chronic HBsAg carriers are listed in Table 2. Serological evidence of acute HAV, HCV, HDV, or HEV superinfection was demonstrated in 27 (45%) of 60 HBsAg carriers with fulminant hepatitis and in 35.6% (32 of 90) of those with nonfulminant hepatitis ($P > 0.2$). Among patients who had no evidence of hepatotropic virus superinfection, the frequency of HBV DNA positivity by dot spot hybridization showed no significant difference between patients with fulminant hepatitis and those with nonfulminant hepatitis. All but one IgM anti-HDV-positive patient were also positive for IgG anti-HDV in titers of less than 1:100, suggesting acute rather than chronic HDV infection (11). Only a few patients with serum HCV RNA were anti-HCV positive by second-generation enzyme immunoassay (Table 2), and all had signal cutoff ratios of less than 2.0. These findings might be compatible with acute HCV infection. However, the possibility of interference of concurrent chronic HBV infection with anti-HCV response cannot be

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TABLE 1. Serodiagnosis of acute non-B hepatotropic virus superinfection in chronic HBsAg carriers

Diagnosis	Test	Assay name, source, and reference(s)	Criterion
Acute hepatitis A	IgM anti-HAV	HAVAB-M (Abbott Laboratories) (6)	Positive IgM anti-HAV
Acute hepatitis C	Anti-HCV	UBI-HCV enzyme immunoassay (United Biochemical, Inc.) (12, 25)	Positive HCV RNA with or without anti-HCV
	HCV RNA	AMPLICOR HCV test (Roche Diagnostic Systems) (17)	
Acute hepatitis D	IgG anti-HDV	Anti-delta (Abbott Laboratories) (20)	Positive IgM anti-HDV with IgG anti-HDV in titers of less than 1:100 (11)
	IgM anti-HDV	Deltassay IgM (Cambridge Biotech, Dublin, Ireland) (22, 23)	
Acute hepatitis E	IgM anti-HEV	ELISA[a] (Genelabs, Inc.) (8)	Positive IgM anti-HEV

[a] ELISA, enzyme-linked immunosorbent assay.

excluded, as observed in patients with concurrent chronic HBV and HCV infection (18).

Only a few cases of acute hepatitis in this study could be attributed to acute HAV superinfection. This finding is in keeping with the previous observations that acute hepatitis A is extremely rare in adults in Taiwan (2), as more than 95% of the adults in the general population have ever been infected with HAV (31). On the other hand, although Taiwan is not an area of endemicity for HEV, sporadic cases of acute hepatitis E not associated with travel to areas of endemicity have been reported elsewhere (14). Regarding the possible transmission routes for HCV and HDV, all patients in this study denied a history of blood transfusion, tattooing, acupuncture, surgery, and dental procedures within 6 months before the onset of acute hepatitis. Other inapparent parenteral routes such as heterosexual transmission should be considered (16).

It is noteworthy that the relative frequency of acute HAV, HCV, HDV, and HEV superinfection showed no significant difference between patients with fulminant hepatitis and those with nonfulminant hepatitis. Furthermore, the relative risk of fulminant hepatitis in HBsAg carriers with acute HAV, HCV, HDV, or HEV superinfection was not significantly different from the risk for those with acute reactivation of chronic HBV infection (Table 2). These data thus highly suggest that viral superinfection with HAV, HCV, HDV, or HEV and acute reactivation of chronic HBV infection might contribute to the development of fulminant hepatitis with a similar risk. It seems that the varied etiology of fulminant hepatitis superimposed upon chronic HBsAg carriers in the previous studies (1, 9, 19, 26, 28, 29) might reflect only the different geographic and ethnic origins of the study patients.

TABLE 2. Results of serological tests for acute fulminant versus nonfulminant hepatitis superimposed upon chronic HBsAg carriers

Serological test	No. of fulminant patients (n = 60)	No. of nonfulminant patients (n = 90)	Odds ratio (95% confidence intervals)	P value[b]
IgM anti-HAV positive	0	2	0.35 (0.00–453.43)	>0.2
HCV RNA positive	11 (1)[a]	19 (2)[a]	1.02 (0.42–2.46)	>0.2
IgM anti-HDV positive	9	7	2.26 (0.73–7.02)	0.16
IgM anti-HEV positive	2	4	0.88 (0.14–5.52)	>0.2
HCV RNA and IgM anti-HDV positive	5 (1)[a]	0	19.17 (1.51–243.04)	0.02
Other				
HBV DNA negative	16	28	1.01 (0.37–2.79)	>0.2
HBV DNA positive	17	30	1.00 (referent)	

[a] Numbers in parentheses indicate patients with positive anti-HCV assay.
[b] Statistical analyses were conducted by the chi-square test with Yates' correction or by Fisher's exact test.

Another important finding of the present study is that 8% of HBsAg carriers with fulminant hepatitis had simultaneous acute HCV and HDV superinfection, compared to none of those with nonfulminant hepatitis (P = 0.02). Our previous study has shown that 0.5 to 1% of HBsAg carriers in Taiwan had serological evidence of concurrent infection with both HCV and HDV (24). The most important clinical implication of the present study is that HBsAg carriers might acquire acute HCV and HDV infection simultaneously from a carrier of HBV, HCV, and HDV and that simultaneous acute HCV and HDV superinfection significantly increases the severity of liver cell damage (Table 2).

Finally, only 1 (3.6%) of 28 HBsAg carriers with fulminant hepatitis was HGV RNA positive (this one also had acute HCV infection), as were 3.3% (3 of 90) of those with nonfulminant hepatitis (two had acute HCV infection and one had acute hepatitis of unidentified cause), suggesting that the role of HGV infection in HBsAg carriers with superimposed acute fulminant or nonfulminant hepatitis is limited, if any.

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