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Nonprogressive Course of Non-A, Non-B Chronic Hepatitis in Multitransfused Hemophiliacs

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Eleven hemophiliacs with chronic liver disease were studied prospectively for 6 yr, with liver function tests and liver biopsies carried out at intervals of 3 yr. The second series of biopsies, compared with the first series, showed continuation of chronic persistent hepatitis in four patients, change to chronic lobular hepatitis in two, and spontaneous improvement of the disease in the four cases who had had chronic active hepatitis characterized by moderate piecemeal necrosis. One patient with active cirrhosis died of liver failure during the follow-up period. Study of the serum and intrahepatic markers for hepatitis B and delta viruses suggests that chronic liver disease is nonprogressive in hemophiliacs who have no intrahepatic viral marker.

A HIGH INCIDENCE of abnormal liver chemistry, especially of high transaminase levels, is found in hemophiliacs transfused with clotting factor concentrates prepared from multiple donors.[1-4] An important step forward in our understanding of the significance of these alterations was recently achieved because of the availability of liver biopsies from 43 patients with persistently elevated transaminase levels.[5-9] The great majority of the biopsies (41 cases) provided histologic evidence for chronic liver disease (CLD), ranging from chronic persistent hepatitis to cirrhosis. A lower but important incidence of CLD (20 cases) was also found among 32 unselected hemophiliacs who were biopsied during massive substitution therapy given to cover surgical procedures.[10]

Even though these studies clearly show that there is a spectrum of histologic abnormalities in hemophiliacs, there are still a number of unanswered questions concerning their clinical significance. The majority of biopsied patients were asymptomatic,[6-9] and there is at the moment no evidence that CLD is a prominent cause of morbidity and death in hemophiliacs.[11] On the other hand, the course of CLD in these patients might be unfavorably influenced by their continuous reexposure to blood-borne viruses and by an unique immunologic situation related to repeated and long-lasting challenge with allogeneic plasma proteins transfused in the concentrates.

Clarification of these points is of paramount clinical importance for their potential impact on the pattern of life of hemophiliacs, as well as on the strategies to be undertaken to prevent and treat CLD. We have chosen to achieve a better understanding of the course of CLD in hemophiliacs by studying 11 patients prospectively and by repeating biopsies at intervals of 3 yr. This report of a 6 yr clinical, biochemical, and histologic follow-up shows that the CLD remained unchanged or was improved in patients with no evidence for hepatitis B virus (HBV) nor delta (δ) virus markets.

MATERIALS AND METHODS

Patients

The 11 patients included in this study (8 with severe hemophilia A and 3 with severe hemophilia B) are those described in a previous report.[6] Their follow-ups were started in 1974 when, during a study of liver function tests (LFT) in 91 hemophiliacs,[1] they were found to have elevated levels (20 IU/liter or more) of aspartate aminotransferase (AST). In 1977, 20 patients with persistently elevated AST at 3 annual visits were considered for liver biopsy. Eleven patients gave informed consent for the procedure, which was performed during substitution therapy with commercial factor VIII or factor IX concentrates.[6] All the patients (identified by numbers 1-11 in our original report[6]) were subsequently followed for LFT and HBV serum markers at least every year, with interviews and physical examinations every 3-6 mo.

Sera and Biopsies

In June 1980, the 10 surviving patients underwent repeat biopsies. Before biopsy, serum samples were tested for HBV markers and with LFT. The latter included AST and ALT (alanine aminotransferase), bilirubin, alkaline phosphatase, and serum protein electrophoresis. Tests for HBsAg, and anti-HB's have been performed since 1974 by radioimmunoassay (RIA) methods (Ausria II - 125 and Ausab Abbott, North Chicago, Ill.) Antibody to hepatitis B core antigen (anti-HBc), hepatitis B *e* antigen (HBeAg) and antibody to HBeAg (anti-HBe) have been determined since 1977 by RIA procedures (Corab and HBe kit Abbott, North Chicago, Ill.) Sera obtained since 1977 have also been screened for antibody to delta antigen (δ-Ag) with a previously described solid-phase RIA.[12]

Percutaneous liver biopsies were obtained through a Tru Cut needle (Travenol Laboratories, Rome, Italy) immediately after the infusion of factor VIII (Kryobulin Immuno, Pisa, Italy) or factor IX (Bebulin, Immuno) concentrates with dosages calculated as previously described.[6] The biopsy specimens were divided into two

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portions. One portion was processed for routine histologic examination of the liver and stained with hematoxylin-eosin and Van Gieson stains. Each biopsy of the 1980 series, as well as the corresponding specimens of the 1977 series, were coded and dispatched for "blind" evaluation to an independent pathologist (Professor P.J. Scheuer, Royal Free Hospital, London, U.K.). The criteria adopted for histologic diagnoses are those established by an International Group of Pathologists.[13]

The second portion of the 1980 biopsy was frozen in liquid nitrogen and tested by direct immunofluorescence (IFL) for intrahepatic HBsAg, HBcAg, and δ-Ag. IFL was performed on 5-μ cryostat sections of the specimens fixed with ether for 5 min, using fluorescein isothiocyanate (FITC) labeled human antisera to HBcAg and δ-Ag and guinea pig FITC antiserum to HBsAg. Details about the IFL procedure and preparation and specificities of the antisera have been reported previously.[14] No follow-up specimen was available from patient 10, who died in 1978; IFL examination was thus limited to the formalin-fixed specimen of the 1977 biopsy.

Diagnostic Definitions

For the purpose of this study, CLD was through to be related to HBV when HBsAg serum positivity was accomplished by evidence of intrahepatic HBsAg and HBcAg. CLD was though to be related to non-A, non-B (nAnB) viruses when the following three conditions were met: no history indicative of drug-induced hepatitis, no intrahepatic IFL expression of HBV markers, and no HBsAg in serum.

RESULTS

Clinical, Biochemical and Serological Findings

Since the beginning of the follow-up study, in no patient was there any deliberate restriction of administration of clotting-factor concentrates. The average annual consumption of factor VIII or IX concentrates per patient was 320 U/kg of body weight (range: 210–518) in 1974 when the study was started; 355 U/kg (range: 181–500) in 1977; and 398 U/kg (range: 198–566) in 1980. There were also no substantial changes in the commercial sources of factor VIII (Immuno, Kabi and Hyland) or factor IX (Immuno or Hyland) concentrates.

Only patient 10 showed clear-cut signs of clinical deterioration. After the diagnosis of active cirrhosis was made in 1977, he was treated with prednisone (0.3 mg/kg daily), with no improvement of symptoms nor liver chemistry. He developed ascites and signs of encephalopathy and died of gastrointestinal bleeding in 1978. He was the only patient found to be persistently HBsAg-positive throughout the study. Other HBV serum markers (tested for since 1977) were anti-HBc and anti-δ, whereas anti-Hbs and anti-HBe were negative (Table 1).

The other 10 patients remained free of severe symptoms, regardless of whether they were not given corticosteroids (patients 1–5, 8, 9, and 11) or were treated with prednisone (patients 6 and 7) for 4 or 12 mo.[6] In no patient did AST, which had been abnormal from 1974 to 1977, remain persistently elevated throughout the next 3-yr period: in two of them (2 and 6), values were within the normal range, while fluctuating levels were seen in the remaining 8 patients. ALT showed a similar pattern of changes, whereas other LFT were usually unchanged or only slightly altered. The AST and ALT values for 1974, 1977, and 1980 are shown in Table 1 to indicate the oscillations in these enzymes. Serum HBsAg remained undetectable in 9 patients, but it has been positive since 1979 in patient 9. All the patients were anti-HBs (except 9) and anti-HBc positive (except 5). None was positive for HBeAg or anti-δ; 3 were positive (2, 3, and 9) for anti-HBe (Table 1).

Liver Biopsies

Table 1 summarizes the histologic findings at the time of the first and second liver biopsies. In the first the independent pathologist (P.J.S.) made a "blind" diagnosis of chronic persistent hepatitis (CPH) for 6 patients (1, 3–5, 8, and 11), of chronic active hepatitis (CAH) for 2 (7 and 9), of transitional forms (CPH/CAH) for 2 (2 and 6) and of cirrhosis for 1 (10). In a few cases his diagnoses were slightly different from those reported by one of us (M.C.) in the previous study.[6] In the second series, he found that the histologic picture remained substantially unchanged for all the patients with CPH, whereas there was an improvement in disease activity, characterized by disappearance of piecemeal periportal nerosis, in the cases originally diagnosed as CAH or CPH/CAH. Accordingly, these last patients now show features of CPH or chronic lobular hepatitic (CLH), namely portal inflammatory infiltration accompanied by rare to diffuse spotty hepatocellular necrosis.

By IFL carried out on fresh tissue, no intrahepatic expression of HBsAg, HBcAg, and δ-Ag was detectable in 10 patients (1–9 and 11). Patient 10 showed HBsAg and δ-Ag, but not HBcAg in formalin-fixed tissue specimens collected in 1977 (see Table 1).

DISCUSSION

In this present study of CLD in 11 hemophiliacs multitransfused with concentrates manufactured from large plasma pools, viral etiology was assumed on epidemiologic grounds and by exclusion of toxic agents. HBV-related CLD was diagnosed by the presence of HBsAg in serum in association with the IFL demonstration of HBV markers in liver specimens, whereas patients lacking these markers were presumed to have nAnB chronic hepatitis.

All our patients with no evidence of intrahepatic HBV markers had nonprogressive CLD, despite their continued challenge with probably infectious blood products. The second series of liver biopsies, compared in blind with the first series by an independent pathologist, showed continuing CPH in 4 cases (4, 5, 8, and 11), a change to chronic lobular hepatitis in 2 (1 and 3), and spontaneous improvement of disease activity in 3 cases with CPH/CAH or CAH characterized by moderate piecemeal necrosis (2, 6, and 7). In patient 9, the pathogenic role of HBV is uncertain despite positive serum HBsAg appearing in 1979 and persisting thereafter, because HBsAg and HBcAg were undetectable in the hepatocytes. He was anti-HBe positive and showed no serum or tissue expressions of the HBV-associated δ-Ag, a specificity associated with a pathogenic defective RNA virus coinfecting HBsAg carriers.[14] In 1977, he showed histologic signs of a mild CAH, which on repeat biopsy appeared to have subsided. This patient might suffer from chronic hepatitis type B, with no intrahepatic expression of HBV and little tendency to progression. This possibility is unlikely because the patient's serum was positive for anti-HBe, a sign of suppressed HBV replication. Rath-

Table 1. Six-Year Follow-up of Liver Function Tests and Histology in 11 Hemophiliacs

Patient	Age (yr) in 1980	Type of Hemophilia	Year of Study	Serum Findings: AST (IU/Liter) (Normal < 20)	Serum Findings: ALT (IU/Liter) (normal < 20)	Serum Findings: HBsAg	Serum Findings: Anti-HBs	Serum Findings: Other HBV Markers	Tissue Findings: Immunofluorescence: Cytoplasm	Tissue Findings: Immunofluorescence: Nucleus	Tissue Findings: Histology: Lobule	Tissue Findings: Histology: Portal Tract	Diagnosis	Histologic Course
			1974	*41**	*39*	Neg	Pos	ND†	ND	ND	ND	ND		
1	21	A	1977	*24*	9	Neg	Pos	ND	ND	ND	Spotty necrosis	Mononuclear infiltration	CPH‡	
			1980	13	10	Neg	Pos	Anti-HBc	Neg	Neg	Necrosis, inflammation, steatosis	Mononuclear infiltration	CLH	Unchanged
			1974	*59*	*50*	Neg	Pos	ND	ND	ND	ND	ND		
2	15	B	1977	*31*	9	Neg	Pos	ND	ND	ND	Minimal piecemeal	Mononuclear infiltration	CPH/CAH	
			1980	18	10	Neg	Pos	Anti-HBc, anti-HBe	Neg	Neg	Rare foci of necrosis	Mononuclear infiltration	CPH	Improved
			1974	*33*	*23*	Neg	Pos	ND	ND	ND	ND	ND		
3	14	A	1977	*22*	5	Neg	Pos	ND	ND	ND	Spotty necrosis	Mononuclear infiltration	CPH	
			1980	10	9	Neg	Pos	Anti-HBc, anti-HBe	Neg	Neg	Spotty necrosis, steatosis	Mononuclear infiltration	CLH	Unchanged
			1974	*71*	*61*	Neg	Pos	ND	ND	ND	ND	ND		
4	16	B	1977	*67*	*59*	Neg	Pos	ND	ND	ND	Spotty necrosis	Mononuclear infiltration	CPH	
			1980	12	*24*	Neg	Pos	Anti-HBc	Neg	Neg	Increased sinusoidal cellularity; steatosis	Mononuclear infiltration	CPH	Unchanged
			1974	*81*	*70*	Neg	Pos	ND	ND	ND	ND	ND		
5	10	A	1977	*38*	6	Neg	Pos	ND	ND	ND	Spotty necrosis	Mononuclear infiltration	CPH	
			1980	*42*	*28*	Neg	Pos	None	Neg	Neg	Spotty necrosis	Mononuclear infiltration	CPH	Unchanged
			1974	*103*	*45*	Neg	Pos	ND	ND	ND	ND	ND		
6	15	A	1977	*40*	24	Neg	Pos	ND	ND	ND	Piecemeal periportal and spotty necrosis	Mononuclear infiltration	CPH/CAH	
			1980	18	16	Neg	Pos	Anti-HBc	Neg	Neg	Spotty necrosis	Mononuclear infiltration	CPH	Improved
			1974	*106*	*55*	Neg	Pos	ND	ND	ND	ND	ND		
7	18	A	1977	*46*	*23*	Neg	Pos	ND	ND	ND	Piecemeal periportal necrosis	Mononuclear infiltration	CAH	
			1980	*52*	*63*	Neg	Pos	Anti-HBc	Neg	Neg	Diffuse necrosis, steatosis	Mononuclear infiltration	CPH	Improved
			1974	*88*	*51*	Neg	Neg	ND	ND	ND	ND	ND		
8	9	A	1977	*67*	49	Neg	Neg	ND	ND	ND	Spotty necrosis	Mononuclear infiltration	CPH	
			1980	*19*	12	Neg	Pos	Anti-HBc	Neg	Neg	Spotty necrosis	Mononuclear infiltration	CPH	Unchanged
			1974	*100*	*130*	Neg	Neg	ND	ND	ND	ND	ND		
9	8	A	1977	*103*	*25*	Neg	Neg	ND	ND	ND	Piecemeal periportal necrosis	Mononuclear infiltration septa, ductular, regeneration	CAH	
			1980	*31*	*20*	Pos	Neg	Anti-HBc, anti-HBe	Neg	Neg	Spotty necrosis	Mononuclear infiltration	CLH	Improved
			1974	*89*	*70*	Pos	Neg	ND	ND	ND	ND	ND		
10	Died in 1978	A	1977	*112*	*37*	Pos	Neg	Anti-HBc, anti-delta	HBsAg	Delta-Ag	Active septa	Fibrosis, mononuclear infiltration, ductular, regeneration	Active cirrhosis	
			1980	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Died
			1974	*41*	*91*	Neg	Pos	ND	ND	ND	ND	ND		
11	50	B	1977	*22*	*188*	Neg	Pos	ND	Neg	Neg	Spotty necrosis; steatosis	Mononuclear infiltration, fibrosis	CPH	
			1980	19	16	Neg	Pos	Anti-HBc	Neg	Neg	Spotty necrosis; steatosis	Mononuclear infiltration, fibrosis	CPH	Unchanged

*Abnormal values are written in italics.

†ND, not done.

‡CAH, chronic active hepatitis; CPH, chronic persistent hepatitis; CLH, chronic lobular hepatitis.

er, we favor the possibility that in 1979 the patient became a "healthy" carrier of HBsAg, with superimposed CLD from an earlier nAnB infection. In the other HBsAg-positive hemophiliac (no. 10), intrahepatic HBsAg and δ-Ag were found in the formalin-fixed biopsy specimen obtained in 1977. Genuine absence of HBcAg is hard to establish since a negative IFL reaction may be due to the effect of sample fixation. After a histologic diagnosis of active cirrhosis made in 1977, he developed liver failure and died 1 yr later.

To what extent are the observations made in this series of patients generally applicable to other hemophiliacs? Our patients had been treated life-long with commercial clotting-factor concentrates widely used in the USA and many European countries and were thus presumably exposed to the same infectious hazards as larger populations of hemophiliacs. They also appear to be a representative sample in terms of age, which might influence CLD through different cumulative exposures to concentrates and thus to blood-borne viruses. This study suggests that in hemophiliacs with nAnB chronic hepatitis, progressive disease is not the rule. Our earlier choice to avoid restricting substitution therapy and to abstain from long-term and widespread use of corticosteroids[6] is thus substantiated by clinical and histologic evidence of low morbidity and nonprogressive disease.

Whether unfavorable development and morbidity of CLD is more frequent in patients with HBV or delta infection remains to be determined. It is remarkable, however, that only 2 of the entire series of 91 hemophiliacs followed since 1974 have died from cirrhosis and that both were HBsAg serum positive (7 HBsAg-positive cases in the entire series). One of these was a patient who was not biopsied because he died from encephalopathy and gastrointestinal bleeding in 1975 at the age of 23; the other was patient 10, who was also δ-Ag-positive. Since preliminary evidence suggests that the delta agent is a major cause of CLD in hemophiliacs,[15] its role in progression of the disease should be evaluated more thoroughly.

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