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Liver disease in haemophiliacs: an overstated problem?

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SUMMARY. Successful percutaneous liver biopsy was carried out on 12 multi-transfused haemophiliacs from the Manchester area with persistently abnormal liver function tests. Only one patient showed evidence of chronic active hepatitis with progression to active micronodular cirrhosis although a further four patients showed some evidence of mild chronic active hepatitis. This represents a much lower incidence of severe histological liver damage than many previous reports and implies that liver biopsy in asymptomatic haemophiliacs may not be indicated as a routine procedure, particularly in the absence of proven therapy. Dynamic liver function tests may prove to be a useful indicator of deteriorating liver function in the otherwise asymptomatic haemophiliac.

The increasing use of plasma products has resulted in an improved quality of life for many haemophiliacs. However, over the past decade there have been reports of a high prevalence of abnormal liver function tests in multitransfused haemophiliacs (Mannucci *et al*, 1975; Hasiba *et al*, 1977; Hilgartner & Giardina, 1977; Levine *et al*, 1977; Preston *et al*, 1978). Liver biopsies carried out in such patients have been reported as showing that up to half the cases are associated with abnormal liver histological appearances including chronic active hepatitis (CAH) (Lesesne *et al*, 1977; Mannucci *et al*, 1978; Preston *et al*, 1978; Spero *et al*, 1978; Schimpf *et al*, 1981).

Liver biopsy in haemophiliacs is an invasive procedure not devoid of risks. Other tests of liver function are available including the measurement of intravenous galactose elimination rate, which is a measure of the functioning liver cell mass (Tengström, 1968), and the plasma disappearance of bromosulphthalein, the kinetics of which reflect hepatocyte perfusion, uptake and biliary canalicular excretion (Häcki *et al*, 1976).

The purpose of this study was to assess the severity of liver disease in haemophiliacs from the Manchester Haemophilia Centre with persistently abnormal hepatic enzyme tests and to

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evaluate the usefulness of non-invasive tests (galactose elimination rate and bromosulphthalein test) as indicators of liver damage.

PATIENTS

All haemophiliacs attending the department were regularly screened at 3–6 monthly intervals for biochemical evidence of hepatic dysfunction.

The criteria for selection for liver biopsy were raised serum aminotransferase levels of greater than 6 months duration, absence of inhibitors to factors VIII or IX, and hepatitis B surface antigen negativity. Patient selection was otherwise random, and independent of the degree of biochemical abnormality, or the type of replacement therapy used previously (i.e. freeze dried preparations or cryoprecipitate).

Thirteen patients underwent liver biopsy. Ten had factor VIII:C levels of less than 0·01 u/ml, two (cases 5 and 8) had factor VIII:C levels of 0·03 u/ml, and one (case 7) had a factor IX:C level of 0·04 u/ml. Case 8 admitted to heavy alcohol consumption and had mild hepatomegaly on examination. The remaining patients were moderate or non-drinkers and had no symptoms or clinical stigmata of liver disease. All patients were considered fit at the time of biopsy and each gave written informed consent.

METHODS

Pre-biopsy haemoglobin level, platelet count, prothrombin time, albumin and total protein levels together with barium swallow and meal examinations (to exclude oesophageal varices) were normal in all selected patients. A 99m Tc sulphur colloid liver and spleen scan was performed in each case before biopsy. Other investigations included serial estimations of aspartate transaminase (AST, SGOT), alanine transaminase (ALT, SGPT), bilirubin, alkaline phosphatase, and gamma glutamyl transferase (GGT). Hepatitis B surface antigen (HGsAg), was screened by Reverse Passive Haemagglutination (RPHA) using 'Hepatest' (Wellcome Laboratories), hepatitis B surface antibody (anti-HBs) by radioimmune assay using 'Ausab' (Abbott Laboratories) and hepatitis B core antibody (anti-HBc) by an Elisa method using 'Corzyme' (Abbott Laboratories).

Alpha-1-antitrypsin levels (A_1AT) and phenotypes, cytomegalovirus, Epstein-Barr virus and toxoplasma antibody titres, hepatitis A virus antibody, smooth muscle antibodies, antimitochondrial antibodies, anti-ds-DNA antibodies and anti-nuclear factor were checked to exclude other causes of hepatitis.

All factor VIII/factor IX replacement products were screened for transaminase and anti-HBc content.

Liver biopsy specimens were examined under light microscopy using conventional histological stains. In addition immunoperoxidase stains were done for detection of HBsAg.

Galactose elimination rate was estimated using the method of Tengström (1968), modified to use a dose of galactose of 500 mg/kg body weight. The plasma disappearance kinetics of bromosulphthalein (BSP) were quantitated using the method of Häcki *et al* (1976).

Biopsy procedure

Immediately prior to biopsy a dose of factor VIII/factor IX calculated to raise the circulating level to 1·0 u/ml was infused, and 20 min later the patients factor level was assayed to ensure an adequate circulating concentration. Percutaneous liver biopsy was then performed with a 'Tru-cut' needle (Travenol Laboratories) under local anaesthesia using aseptic technique. Replacement therapy was given at 8-hourly intervals, maintaining the factor VIII/factor IX level at a minimum of 0·5 u/ml for 36 h and then 0·2 u/ml for a further 36 h. Oral tranexamic acid (500 mg 8-hourly) was prescribed for 5 d over the post biopsy period.

Before discharge from hospital, traumatic hepatic haematomata were excluded by ultrasonic liver scan. The average duration of stay in hospital was 4·5 d and no complications associated with the biopsy procedure were observed in any patient.

RESULTS

Out of a total of 153 haemophiliacs attending the Department of Clinical Haematology, Manchester Royal Infirmary, during 1982, 79 (52%) were found to have abnormal liver function tests.

Liver biopsy specimens were obtained in 12 cases and were reviewed by three independent pathologists. The histological diagnoses are recorded in Table I.

Splenomegaly was noted in eight patients (61%) on 99m Tc sulphur colloid scan and three patients (23%) showed hepatomegaly.

Table I compares liver function tests taken immediately before biopsy with histological diagnosis. Values in parentheses indicate the maximum recorded pre-biopsy level for each individual. The severity of histological damage was unrelated to: (i) the degree of transaminase elevation; (ii) the type, quantity and duration of replacement therapy used previously; and (iii) previous history of clinical hepatitis (reported in cases 1 and 11).

When comparing cases 1–5 (non-specific histological changes) with cases 6–12 (Table I), no statistically significant difference was found between these two groups when comparing the bilirubin, AST, ALT, Alkaline phosphatase and GGT levels either at the time of biopsy or the highest recorded pre-biopsy levels (Mann-Whitney U Test).

A_1AT studies showed twice the expected level of incidence of MS and MZ heterozygote phenotypes, these being present in four patients with either mild chronic active hepatitis or chronic active hepatitis with micronodular cirrhosis (cases 8–12).

The results of the hepatitis serological markers are shown in Table II. All patients were screened for the presence of HBsAg prior to biopsy. Retrospectively, however, case 11 was found to be positive for anti-HB_c IgM. Nine out of the 13 cases (69%) were found to be positive for anti-HBs and seven out of the 13 (54%) positive for anti-HB_c. None of the 12 liver biopsies showed the presence of HB_sAg in the liver sections using immunoperoxidase stains.

Table II shows the results of the dynamic liver function tests performed at the time of biopsy. All five of the patients (cases 1–5) with essentially normal liver histology had normal galactose elimination rates whereas five of the seven patients (cases 6, 8, 10, 11 and 12) with histological changes showed reduced galactose elimination rates. Four of the five patients

Table I. Comparison of liver function tests with histological diagnoses

	Age	A_1 AT phenotype	Bilirubin (μmol/l)	AST (I.U./l)	ALT (I.U./l)	Alk. phos. (I.U./l)	GGT (I.U./l)	Histology
1	30	M	16 (20)	39 (142)	95 (407)	35 (35)	19 (19)	Non-specific changes
2	32	M	20 (29)	30 (66)	71 (156)	53 (67)	23 (23)	Non-specific changes
3	25	M	13 (18)	42 (194)	99 (625)	75 (75)	36 (145)	Non-specific changes
4	26	M	12 (17)	33 (47)	43 (178)	51 (75)	17 (31)	Non-specific changes
5	24	M	25 (29)	35 (64)	82 (148)	53 (67)	31 (42)	Non-specific changes
6	23	M	21 (30)	56 (117)	141 (308)	48 (64)	33 (35)	Chronic lobular hepatitis
7	13	M	12 (12)	504 (504)	380 (380)	134 (135)	13 (15)	Chronic persistent hepatitis
8	21	M	14 (19)	59 (59)	72 (193)	55 (123)	25 (46)	Mild chronic active hepatitis
9	22	MZ	11 (11)	77 (77)	169 (179)	100 (100)	163 (165)	Mild chronic active hepatitis
10	15	MZ	25 (60)	55 (85)	55 (97)	196 (256)	27 (27)	Mild chronic active hepatitis
11	40	MS	5 (26)	26 (650)	26 (1490)	73 (140)	22 (80)	Mild chronic active hepatitis
12	42	MS	20 (20)	40 (40)	65 (261)	61 (94)	53 (53)	Chronic active hepatitis with progression to active micronodular cirrhosis
13	28	M	14 (17)	48 (60)	228 (228)	150 (150)	33 (33)	Failed biopsy
Normal values			<22	<45	<40	<100	<65	

Results in parentheses indicate highest recorded values. Histological definitions: Non-specific changes: Focal mild inflammatory cell infiltrate in the portal tracts and liver parenchyma with focal liver cell necrosis. Chronic lobular hepatitis: Predominantly intralobular inflammation and necrosis, as defined by the International Group (1977). Mild chronic active hepatitis: Dense chronic inflammatory cell infiltrate in portal tracts with mild focal but definite piecemeal necrosis (together with or without mild lobular activity).

Table II. Hepatitis markers and dynamic liver function tests

	HGsAg	Anti-HB_s	Anti-HB_c	Anti-HB_c IgM	Galactose $T\frac{1}{2}$ (min)	BSP K_1 (%/min)	BSP K_1 (%/min)	BSP K_2 (%/min)
1	Neg	Neg	Neg	Neg	10	–	13·9	–
2	Neg	Pos	Pos	Neg	10	14·4	10·7	6·6
3	Neg	Neg	Neg	Neg	12	21·7	13·9	4·5
4	Neg	Pos	Neg	Neg	16	19·25	10·3	3·5
5	Neg	Neg	Neg	Neg	16	13·0	9·5	3·6
6	Neg	Pos	Pos	Neg	20	9·9	6·2	2·7
7	Neg	Pos	Pos	Neg	10	–	–	–
8	Neg	Pos	Neg	Neg	25	14·0	8·5	4·5
9	Neg	Pos	Pos	Neg	11	21·3	10·8	5·6
10	Neg	Pos	Pos	Neg	25	13·5	9·9	4·95
11	Neg	Pos	Pos	Pos	40	13·5	8·7	3·6
12	Neg	Neg	Neg	Neg	22	4·4	10·7	6·6
13	Neg	Pos	Pos	Neg	24	9·1	5·5	3·5
Normal values					<17	14·3±1·5	12·6±1·6	5·3±1·9

(cases 8, 10, 11 and 12) with CAH (albeit very mild in four cases) had abnormal galactose elimination rates whereas only case 6 in the non CAH group showed a mildly prolonged galactose $T\frac{1}{2}$. Patient 12 (with CAH and micronodular cirrhosis) also had a markedly abnormal K_1 value.

DISCUSSION

Fluctuating abnormalities in liver function tests are well recognized in multitransfused haemophiliacs. A 52% rate of abnormal LFTs in haemophiliacs who regularly attend the Manchester Haemophilia Centre is in keeping with previous reports. Studies have also indicated a high incidence of abnormal hepatic histology in these patients with CAH and cirrhosis being found in between one third (Schimpf *et al*, 1981; Spero *et al*, 1978) and one half (Lesesne *et al*, 1977; Mannucci *et al*, 1978; Preston *et al*, 1978) of haemophiliacs biopsied.

In our study only one patient had histological evidence of severe CAH with cirrhosis. A further four patients showed changes of early and mild CAH. The natural history of these cases remains to be established. Although this is a relatively small survey it represents a much lower incidence of severe histological liver changes than most previous reports (Lesesne *et al*, 1977; Mannucci *et al*, 1978; Preston *et al*, 1978; Spero *et al*, 1978; Schimpf *et al*, 1981).

Our patients were all multi-transfused haemophiliacs selected on the basis of abnormal liver enzyme tests. The degree of abnormality typically fluctuated over the period of

observation prior to biopsy but we have no reason to suspect that our patient population had been sheltered from any agent implicated in the pathogenesis of their liver dysfunction. No correlation was found between the liver histological appearances or liver function tests and the duration, total dosage, or nature of the replacement blood products.

The results of this study suggest that the measurement of dynamic liver function tests (particularly the galactose elimination rate) may be useful in the detection of early hepatic dysfunction in multitransfused haemophiliacs. If the plasma disappearance curve of an intravenous dose of BSP is plotted, the initial corrected disappearance rate (K_1) reflects hepatic blood flow and cell perfusion and the uptake of BSP. The final component (K_2) reflects hepatic elimination of BSP, i.e. biliary canalicular function. K_i is the uncorrected initial disappearance rate and is thought to represent a sensitive index for the detection of liver disease (Häcki *et al.* 1976). Our results suggest that while canalicular function remains normal, the predominant lesion in haemophiliacs appears to be one of reduced functioning liver cell mass as measured by the galactose elimination rate and to a lesser extent impaired hepatocyte perfusion and uptake as measured by the BSP K_1 values.

Case 11 was found to be positive (retrospectively) for anti-HBc IgM. The clinical significance of this result remains in doubt although it may imply that the patient had, or had recently had, active hepatitis B virus replication.

It has been suggested (Hodges *et al.* 1981) that persons with the MZ heterozygous form of partial alpha$_1$-antitrypsin deficiency have an increased prevalence of cryptogenic cirrhosis and CAH. The number of patients in this study is small but our findings are in general agreement in that all four heterozygotes (cases 9–12) had either mild CAH or cirrhosis. We are unaware of any published results as to the frequency of MS and MZ heterozygotes in haemophiliacs. In the U.K. Caucasian population, MS represents 9% and MZ 3% of the population. In this small survey, MS and MZ both represent 16% and this compares with figures of 12% and 8% respectively for a series of 52 haemophiliacs in the Sheffield Region (Dr Milford Ward, personal communication).

In this study only one patient was found to have CAH with progression to micronodular cirrhosis. Four other patients had only mild CAH. We suggest that the true incidence of severe histological liver abnormality in multitransfused haemophiliacs may be less than previously reported but similar to the more recent results of 115 liver biopsies carried out world wide (Aledort *et al.* 1981) where the incidence of CAH and cirrhosis was 16%. It is our opinion that routine percutaneous liver biopsy is not indicated at the present time in asymptomatic haemophiliacs with abnormal liver function tests as proven therapy is not available and the natural history of these liver changes has yet to be elucidated. However, advances in the treatment of CAH may influence this policy in the future and biopsy may be indicated where clinical signs and symptoms or other parameters suggest deteriorating liver function. Dynamic liver function tests, particularly the galactose elimination rate, may be particularly useful in this respect.

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