AETIOLOGY OF ACUTE HEPATITIS IN MALAYSIA

DORA S.K. TAN, M., DIMITRAKAKIS**, M. ZAINI RAHMAN, RANDEL FANG, DAVE COLLETT*, OOI BENG GEOK and I.D. GUST**

Institute for Medical Research, Kuala Lampur, *Department of Statistics, National University of Malaysia, Bangi, Selangor, Malaysia and **Fairfield Hospital, Melbourne, Australia.

INTRODUCTION

In Malaysia, the diagnosis of acute viral hepatitis in patients presenting with jaundice is complicated by the relatively high incidence of leptospirosis. To determine the aetiology and relative frequency of different agents in the aetiology of acute hepatitis, a group of 145 patients with acute liver disease was tested for serological evidence of infection with hepatitis A virus (HAV), hepatitis B virus (HBV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) infection and leptospirosis.

MATERIALS AND METHODS

Human serum: Single serum specimens from 145 icteric patients, admitted to hospital in various parts of Malaysia with clinical and biochemical evidence of hepatitis were examined for IgM antibody to hepatitis A virus and hepatitis B virus and for heterophile antibody to EBV. A subset of 102 sera was tested for IgM antibody to CMV. Convalescent sera were available from 64 patients and a diagnosis of leptospirosis was made by examining acute and convalescent sera for significant rises in the titres of leptospiral agglutinins. HBsAg and anti-HBs were determined on 117 sera.

Sera from 178 apparently normal subjects (staff of the Institute for Medical Research, Kuala Lumpur) were tested for levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and bilirubin. These values were compared with the values obtained on sera from the jaundiced patients. All sera were stored at –20°C before testing.

HAV and HBV: Anti-HAV IgM and anti-HBc IgM were detected by enzyme immunoassay using commercial kits Havab and Cozyme M (Abbott Laboratories, Chicago, Ill., U.S.A.). Most sera were also tested for HBsAg and anti-HBs by the solid-phase radioimmunoassay (SPRIA) using commercial reagents, Ausria and Ausab from the same manufacturers.

CMV infection: Anti-CMV IgM was detected by EIA using the commercial reagent Enzynost, with anti-human IgM conjugate (Behringwerke AG, Marburg, W. Germany).

EBV: Heterophile antibodies which are excellent markers of infection with EBV were detected by the Paul-Bunnel (1932) and Davidsohn's (1937) methods using horse red blood cells.

Leptospirosis: The microscopic agglutination (MA) test was employed for the diagnosis of leptospirosis, using live Patoc 1 strain (*L. biflexa* species), a genus-specific antigen. Paired sera were examined for significant rise in titres of leptospiral agglutinin.

Liver function tests: The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined according to the methods recommended by the International Federation of Clinical

The study was supported by the National Council for Scientific Research and Development of Malaysia.

Chemistry (Bergmeyer 1976, 1977 and 1980) using Tris buffer, reaction temperature of 37°C and five-minute analysis time. The analyses were done using the Abbott Biochromatic Analyser ABA 100. The ABA 100 was also used to assay serum bilirubin. The method used is an adaptation of the diazo method of Powell (1944).

RESULTS AND DISCUSSION

Table 1 shows the aetiology of the disease in those patients who were tested. Of 145 patients examined, 16 (11%) were confirmed as suffering from hepatitis A on the basis of detection of hepatitis A specific IgM, and a further 6 (4.1%) as suffering from acute hepatitis B on the basis of detection of high titres of anti-HBc IgM. Of 102 patients tested for evidence of acute infection with CMV only 1 was positive and no patient had evidence of acute infection with EBV. Of the 64 patients in whom acute and convalescent sera were available, 16 (17.2%) had evidence of leptospirosis. If one extrapolates these figures to the whole group, approximately 67.9% of cases of hepatitis in Malaysia are non-A, non-B.

It may be observed that the occurrence of leptospiral hepatitis with frank icterus in Malaysia as in other tropical and subtropical countries is relatively low (Tan, 1979), perhaps because infection is widespread and the local population has already been immunized by subclinical or anicteric infection experienced early in life (Tan *et al.*, unpublished).

A total of 117 patients were tested for HBsAg, anti-HBs and anti-HBcAg. HBsAg was detected in 10 (8.5%), anti-HBs in 27 (23.1%) and anti-HBc IgM in 6 (5.1%).

A variety of patterns of HBV markers were detected which enabled us to define several clinical categories (Table 2).

Two (1.7%) of the 117 patients with acute HBV infection were negative for HBsAg and would have been missed if only HBsAg-positive sera were tested for IgM anti-HBc. Several workers (Feinman *et al.*, 1981; Chau *et al.*, 1983; Lavarini, 1983) had pointed out the usefulness of the IgM anti-HBc in (a) the detection of subclinical HBV infection and infection accompanied by low or undetectable levels of HBsAg, (b) differentiation of acute and chronic infections, (c) defining prognosis as it has been shown that there is an association between the absence of IgM anti-HBc at the onset of hepatitis and the persistence of HBsAg at follow-up.

Low levels of IgM anti-HBc can be detected in patients recovering from acute hepatitis B for up to 18 months after onset of illness and have been detected in some chronic carriers with chronic active hepatitis or primary liver

Table 1

Aetiology of acute hepatitis in Malaysia.

Aetiology	Patients	No. Pos.	Percent	S.E. (%)
Hepatitis A	145	16	11.0	2.6
Hepatitis B	145	6	4.1	1.6
CMV Infection	102	1	1.0	1.0
Infectious mononucleosis	145	0	0	0.0
Leptospirosis	64	11	17.1	4.7

Table 2

Patterns of HBV markers in 117 patients with acute hepatitis.

HBsAg	Anti-HBS	Anti-HBc IgM	No. (%)	Interpretation
+	−	−	5 (4.3)	Hepatitis B (late incubation period)
+	−	+	4 (3.4)	Hepatitis B (acute infection)
−	−	+	2 (1.7)	Hepatitis B (recent acute infection)
−	+	−	26 (22.2)	Past infection with HBV
+	+	−	1 (0.9)	Chronic carrier of HBV
−	−	−	79 (67.5)	Non-B, hepatitis

Table 3

Liver function test results of patients with acute hepatitis compared with normal subjects.

Group(n)	ALT		AST		Serum Bilirubin	
	$\bar{x}$ (range)	S.D.	$\bar{x}$ (range)	S.D.	$\bar{x}$ (range)	S.D.
Normal (n=178)	25.6 (2-155)	21.2	24.2 (7-81)	11.2	0.9 (0.3-2.0)	0.3
Hepatitis A (n=15)	576.5 (19-1610)	546.6	632.5 (48-2660)	844.7	7.6 (1.2-14.1)	7.6
Hepatitis B (n=6)	526.0 (34-1428)	665.2	184.4 (7.5-478)	194.7	2.9 (1.4-6.5)	1.9
Leptospirosis (n=6)	42.5 (18-67)	20.0	44.7 (23-66)	18.5	7.1 (2.4-20.5)	7.3
NANB (n=25)	61.6 (9-250)	54.7	70.0 (16-250)	58.4	3.5 (1.0-13.8)	3.2

ALT = Alanine aminotransferase
AST = Aspartate aminotransferase

carcinoma as well as in some healthy carriers. (Roggendorf *et al.*, 1981).

The levels of the transaminases and bilirubin in the sera of the patients with viral hepatitis were compared with patients with leptospirosis and with normal subjects (Table 3). Both patients with hepatitis A and B had mean ALT and AST values of over 500 IU indicating severe liver damage, whereas in leptospirosis and non-A, non-B hepatitis the levels were much lower. Serum bilirubin levels, on the other hand, were higher in patients with hepatitis A and leptospirosis (about 7mg%) than in those with hepatitis B (2.9%), the normal mean value being 0.9 mg%. These figures are consistent with the general clinical and pathological stage of the respective illnesses.

SUMMARY

Icteric patients with clinical and biochemical evidence of liver disease, admitted into various hospitals in Malaysia, were investigated to determine the cause of their infection.

Of these patients, 11.0% (16/145) were found positive for IgM anti-HAV (EIA), 4.1% (6/145) for IgM anti-HBc (EIA), 1.0% (1/102) for IgM anti-CMV (ELISA), 17.2% (16/64) for rising titres of leptospiral agglutinin, and none for heterophile antibody of EBV. Hepatitis NANB accounted for 67.9% of cases.

The mean serum transaminases (ALT and AST) values in patients with hepatitis A and B were higher (more than 500IU) than in patients with leptospirosis or non-A, non-B hepatitis, whereas serum bilirubin levels were higher in patients with hepatitis A and leptospirosis than in patients with hepatitis B.

ACKNOWLEDGEMENTS

The authors are grateful to the National Council for Scientific Research and Development for their support and their research grant for this study (MPKSN/C/8/004/A(45)). They also thank the Director of the Institute for Medical Research for permission to publish this paper.

REFERENCES

BERGMEYER, H.U., BOWERS, G.K., JR., MORDER, M. and MOSS, D.W., (1976). Provisional recommendations on IFCC method for the measurement of catalytic concentration of enzymes. *Clin. Chim. Acta., 70* : F19

BERGMEYER, H.U. and HORDER, M., (1980). IFCC method for the measurement of catalytic concentration of enzymes, *ibid, 105* : 147F

BERGMEYER, H.U., HORDER, M. and MOSS, D.W., (1977). Provisional recommendations on IFCC method for the measurement of catalytic concentration of enzymes, *ibid, 80* : 21.

CHAU, K.H, HARGIE, M.P., DECKER R.H., MUSHAHWAR, I.K. and OVERBY, L.R., (1983). Serodiagnosis of recent hepatitis B infection by IgM class anti-HBc. *Hepatology, 3* : 142.

DAVIDSOHN, I., (1937). Serologic diagnosis of infectious mononucleosis. *JAMA, 108* : 289.

FEINMAN, S.V., OVERBY, L.R., BERRIS, B., CHAU, K.H., SCHABLE, C.A. and MAYNARD, H.E., (1982). The significance of IgM antibodies to hepatitis B core antigen in hepatitis B carriers and hepatitis B-associated chronic liver disease. *Hepatology, 2* : 795.

LAVARINI, C., FARCI, P., CHIABERGE, E., VEGLIO, V., GIACOBBI, D., BEDARIDA, G., SUSANI, G. *et al.*, (1983). IgM antibody against hepatitic B core antigen (IgM anti-HBc): diagnostic and prognostic significance in acute HBsAg positive hepatitis. *Brit. Med. J., 287* : 1254.

PAUL, J.R. and BUNNELL, W.A., (1932). The presence of heterophile antibodies in infectious mononucleosis and in other diseases. *Amer. J. Med. Sci., 183* : 90.

POWELL, W.N., (1944). A method for the quantitative determination of serum bilirubin with the photoelectric colorimeter. *Amer. J. Clin. Path. Tech. Section, 8* : 55.,

ROGGENDORF, M., DEINHARDT, F., FORSNER, G.G., SCHEILD, R., BAYERI, B. and ZACHOVAL, R., (1981). Immunoglobulin M antibodies to hepatitis B core antigen: evaluation of enzyme immunoassay for diagnosis of hepatitis B virus infection. *J. Clin. Microbiol., 13* : 618.

TAN, DORA, S.K., (1979). Leptospirosis in West Malaysia - epidemiology and laboratory diagnosis. *Malaysian J. Path., 2* : 1.