PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 161, 322-325 (1979)

Differential Serodiagnosis of Sporadic Acute Viral Hepatitis (40545)[1]

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Etiologic identification of the specific infectious agent associated with acute viral hepatitis is important for clinical management of the patient and for prevention of the spread of the disease. In sporadic cases, in contrast to point-source epidemics, epidemiologic histories are not reliable for etiologic conclusions, since histories normally do not differ in hepatitis B surface antigen (HBsAg)-positive and HBsAg-negative patients (1, 2). Recent advances in the serodiagnosis of hepatitis B virus (HBV), hepatitis A virus (HAV), Epstein–Barr virus (EBV), and cytomegalovirus (CMV) infections have confirmed the heterogeneity of HBsAg-negative sporadic hepatitis (3, 4). These studies inferred, by exclusion of HAV and HBV, that one or even two non-A, non-B hepatitis viruses were responsible for transfusion-associated disease, as well as sporadic cases in the general population (5–7). Further studies have shown that the epidemiologic and clinical characteristics of the non-A, non-B viruses are different from that of HAV (8). In the present paper we report the results of a study of 222 adult patients hospitalized with acute sporadic viral hepatitis. The study was designed to estimate the relative proportion of HAV, HBV, and non-A, non-B disease in patients hospitalized over a 6-month period in Athens, Greece.

Materials and methods. Patients. The study included 222 sporadic acute viral hepatitis cases consecutively admitted to the Infectious Diseases Hospital of Athens from October 1976 to March 1977. There were 124 (56%) males and 98 (44%) females included in the study; about one-third (71) were young adults, 20–29 years old. A personal interview was used to complete a detailed questionnaire, which included data on prior hepatitis, known personal contacts, or possible exposure (sexual, occupational, hospitalization, transfusion, illicit self-injection, etc.) within a 6-month period preceding illness. The diagnosis of hepatitis was based on epidemiologic and medical history, typical clinical symptoms and signs, and characteristic laboratory findings. Sera were collected on the days of admission and day of discharge from the hospital. A stool sample was collected from the patients as early as possible (1–4 days) after their admission.

Serologic tests. The serum from each patient was tested for HBsAg by reversed passive hemagglutination (RPHA) (9). A total of 153 were found positive. A more intensive study was done in 26 out of the 69 negative for HBsAg by PHA. They were selected because sera at admission and discharge from the hospital as well as feces were available. These sera were further tested by radioimmunoassay for HBsAg, antibody to HBsAg (anti-HBs) (10), antibody to core antigen (anti-HBc) (11), and antibody to HAV (anti-HAV) (12). Extracts of feces were analyzed for HAV by radioimmunoassay (12). Those sera which were anti-HAV positive were further tested for IgM anti-HAV by a modification of the method described by Kalimo *et al.* (13). This method employed antibodies specific for the μ determinant of IgM and for the γ determinant of IgG as a solid-phase reagent. Anti-human μ or anti-human γ were coated on separate polystyrene 5-mm beads. A dilution of a serum to be tested was then reacted with each bead in order to bind IgM- and IgG-class immunoglobulins, respectively. The beads were then reacted with a concentrate of hepatitis A virus, followed by ^{125}I-labeled anti-HAV. The amount of radiolabeled antibody bound was in direct proportion to the amount of either anti-HAV-specific IgM or IgG in the original serum. The assay will be described in more detail elsewhere. Sera were also analyzed for anti-CMV

[1] This study was supported by a grant from the Ministry of Social Services of Greece.

0037-9727/79/070322-04$01.00/0

by complement fixation (14) and for IgM anti-EBV by indirect immunofluorescence (15).

Results. HBsAg was detected by PHA in the acute-phase serum of 153 (69%) cases. The presence of HBsAg could be associated with acute type B hepatitis or a carrier state of HBV. No prior serology was available to assess the possibility of a long-term carrier state. It was recognized that HBsAg-positive carriers may develop type A or non-A, non-B hepatitis; however, because of their association with hepatitis B the sera were not studied further. The results of the serologic analyses of 26 cases negative for HBsAg by PHA are shown in Table I. Anti-HAV was detected in 25 out of 26 cases. This suggests current or prior exposure to HAV in virtually all patients. A significant rise of anti-HAV titer could be shown in only one of these 25 cases. This suggests that for these patients hospitalized for hepatitis, on the day of admission the titer of anti-HAV had already reached a high titer and did not substantially change during hospitalization. Anti-HAV IgM was demonstrated in 9 cases (patients 1–9, Table I). HAV was detected in the feces of 3 out of these 9 cases (patients 1, 4, and 9). These findings are consistent with a diagnosis of hepatitis A in all 9 patients.

HBsAg was detected by RIA in 4 cases (No. 10, 12, 16, 19). High titers of anti-HBc in the absence of HBsAg or anti-HBs in both the acute and discharge serum were detected in 6 other cases (No. 11, 13–15, 17, 18). The serology of these 10 cases is consistent with hepatitis type B, 4 having detectable HBsAg and 6 having only anti-HBc.

The remaining seven cases (Nos. 20–26) were characterized as hepatitis non-A, non-B, since all of them had serologic evidence of past infection with both HAV (anti-HAV IgG) and HBV (anti-HBs and anti-HBc). None of the patients demonstrated anti-EBV IgM, or showed seroconversions to CMV.

None of the 9 hepatitis A cases had a

TABLE I. SEROLOGIC INVESTIGATION OF SERA AND FECES FROM SPORADIC ACUTE VIRAL HEPATITIS CASES AMONG ADULTS IN GREECE.

Patient No.	Anti-HAV	HAV in stools	HBsAg (RIA)	Anti-HBs	Anti-HBc	Transfusion or iatrogenic[a]	Diagnosis	
							Current	Past
1	IgM	+	−	+	+	—	A	B
2	IgM	−	−	−	+	—	A	B
3	IgM	−	−	−	−	—	A	—
4	IgM	+	−	+	+	—	A	B
5	IgM	−	−	−	−	—	A	—
6	IgM	−	−	−	−	—	A	—
7	IgM	−	−	+	+	—	A	B
8	IgM	−	−	−	−	—	A	—
9	IgM	+	−	+	+	—	A	B
10	IgG	−	+	−	+	Iatr.	B	A
11	IgG	−	+	−	+	Iatr.	B	A
12	IgG	−	+	−	+	Iatr.	B	A
13	IgG	−	+	−	+	Iatr.	B	A
14	—	−	−	−	+	Iatr.	B	—
15	IgG	−	−	−	+	Iatr.	B	A
16	IgG	−	−	−	+	—	B	A
17	IgG	−	−	−	+	—	B	A
19	IgG	−	−	−	+	—	B	A
	IgG	−	−	−	+	—	B	A
20	IgG	−	−	+	+	Transf.	non-A non-B	A + B
21	IgG	−	−	+	+	Transf.	non-A non-B	A + B
22	IgG	−	−	+	+	Iatr.	non-A non-B	A + B
23	IgG	−	−	+	+	—	non-A non-B	A + B
24	IgG	−	−	+	+	Iatr.	non-A non-B	A + B
25	IgG	−	−	+	+	Iatr.	non-A non-B	A + B
26	IgG	−	−	+	+	—	non-A non-B	A + B

[a] History of blood transfusion or possible iatrogenic transmission during the last 6 months.

history of blood transfusion or possible iatrogenic transmission. In contrast, such a history was found in 5 of the 7 hepatitis type non-A, non-B patients and in 6 of the 10 hepatitis type B patients. Hepatitis type A patients were younger than either type B or type non-A, non-B patients. The mean ages were 19.5, 32.5, and 40.0, respectively. The clinical course as well as the biochemical findings (transaminases and bilirubin) did not differ significantly among the three types of viral hepatitis.

Discussion. Recent studies (4–7) have found that the frequency of various types of acute viral hepatitis in sporadic adult cases varies according to the method of selection of the patients, the geographic area, and the serologic techniques used for diagnoses. The present investigation indicates that in Greece almost 80% of the acute sporadic cases in adults are caused by HBV infection. Much lower estimates have been reported for Germany, 60% (7); USA, less than 55% (6); and Australia, less than 40% (4). Minor differences in methodologies were used in these studies; however, the difference appears to be attributed mainly to the higher prevalence of HBV in Greece. This is consistent with the reports of HBsAg carrier rates and evidences of past HBV infection, which are substantially higher in Greece than in Western Europe, USA, or Australia (16). The observed high proportion of HBV infections may be partly due to the low relative frequency of hepatitis A in Greek *adults*, since most of them have been exposed in *childhood* (17). In our study only 11% of the adult cases were identified as hepatitis A disease. In contrast, there were 43% in Australia, 20–25% in USA, and 17% in Germany. In all studies, hepatitis A occurred in younger patients more frequently than either type A or non-A, non-B hepatitis.

It appears from the studied sample that about 9% of sporadic adult acute viral hepatitis cases are caused by other than A or B viruses. Similar results have been reported by Locarnini *et al.* (4), while Dienstag *et al.* (6) concluded that in the USA non-A, non-B hepatitis may comprise some 20–25% of the cases. In accordance with other reports none of our hepatitis cases could be attributed to EBV or CMV (3–7).

Several other conclusions can be drawn from the results of the present investigation. HAV could be detected in feces collected at admission from three out of nine hepatitis A cases. Thus it appears that HAV is not excreted in detectable quantities from most of hepatitis A patients at the time of hospital admission. This supports the hypothesis that communicability of the disease decreases significantly when jaundice appears (18). It also minimizes the potential use of HAV detection in feces as a reliable method for etiologic diagnosis. We were unable to demonstrate increases in titer of anti-HAV in 25 of the 26 paired sera. Thus, the detection of anti-HAV IgM appeared to be a more sensitive and specific method for the diagnosis of hepatitis A at the acute phase of the disease.

Our data indicate that opportunity for parenteral exposure was as frequent in hepatitis B (6/10) as in non-A, non-B (5/7) cases. In contrast, such a history was not present in any of the hepatitis A cases studied. This further stresses the epidemiologic differences between A and non-A, non-B hepatitis (8). However, methods for direct serologic identification of the non-A, non-B virus are necessary to determine the extent of its carrier rate and possible association with chronic liver disease.

Summary. The frequency of the various types of acute sporadic viral hepatitis was studied in a sample of 222 consecutively hospitalized, adult patients. Sera at admission and discharge from the hospital as well as feces were available in 26 out of the 69 cases negative for hepatitis B surface antigen (HBsAg) by passive hemagglutination assay. These were selected for more intensive serologic investigation by solid-phase radioimmunoassay methods. IgM antibodies to HAV (anti-HAV) were detected in 9 cases. In 3 (33%) of them HAV was demonstrated in feces. In 10 cases evidence of current hepatitis B virus (HBV) infection was demonstrated. The remaining 7 cases were characterized as non-A, non-B hepatitis by exclusion because of serologic evidence of past HAV and HBV infection. It appears from the studied sample that in Greece almost 80% of the acute sporadic cases among adults are caused by HBV, 11% by HAV, and about 9% by non-A, non-B virus.

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Received January 22, 1979. P.S.E.B.M. 1979, Vol. 161.