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Hepatitis Viral Markers in Patients Undergoing Primary Liver Transplants

JESSICA H. LEWIS, MD, LOBNA EL-ASHMAWY, MDCHB, GLENN E. RAMSEY, MD, FRANKLIN A. BONTEMPO, MD, MAYA ROCHLANI, MD, ANTHONY J. DEMETRIS, MD, DAVID H. VAN THIEL, MD, and THOMAS E. STARZL, MD, PhD

The purpose of the study was to determine the prevalence in liver transplant (OLTx) patients of the hepatitis markers (anti-A, anti-B, anti-C, anti-D and HB_sAg) and the interrelationships between markers and patients' sexes, ages, dates of transplant, clinicopathological diagnoses, and short-term survivals. Slightly more than half of the patients were male. Anti-A and anti-B were about evenly distributed between male and female. Anti-C, anti-D, and HB_sAg were far more common in males. Age and year of transplant showed only a moderate increase in anti-A with increasing age. Anti-A was found in 57% of all patients, anti-B in 18%, anti-C in 17%, and HB_sAg in 17%. Anti-D was tested only in patients who were positive for anti-B or HB_sAg and occurred in 21 (11%) of 185. The poorest short-term survival occurred in males who showed both anti-A and HB_sAg.

KEY WORDS: hepatitis viruses; anti-HAV; anti-HBV; anti-HCV; anti-HDV; hepatitis B surface antigen; liver transplants.

Liver disease of sufficient severity to necessitate liver transplant may arise from a variety of irreversible diseases, including acute or chronic infection with one or more of the hepatitis viruses. In an attempt to assess the role of these viruses, we have determined their prevalence in baseline plasma specimens from 573 patients undergoing primary orthotopic liver transplant (OLTx). These plasma samples were residual from coagulation studies, and their use was approved by the University of Pittsburgh Institutional Review Board. Because the hepatitis C testing was done before the test systems were approved by the Food and Drug Administration (FDA), the results were treated as experimental and not reported to patients' physicians.

Plasma samples had been stored at −70° C for up to eight years (1982–1989). No patient had received FK705 before the blood was tested. Antibodies to hepatitis A, B, and C and the antigen, HB_sAg, were assayed in all samples. Anti-D (delta) was assayed only in those positive for HB_sAg and/or anti HB_sAg (anti-B).

The primary aim of the study was to determine the prevalence of these markers. A relationship between positive markers or marker patterns was sought with sex, age, year of transplant, clinicopathological diagnosis, and short-term survival.

MATERIALS AND METHODS

Plasma Samples. These were "left over" from plasma taken for coagulation studies on either the baseline or first operative (preliver excision) sample and stored frozen at −70° C for one to eight years. Because the samples were left over, the project was approved by the University of

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From the Central Blood Bank, Pittsburgh, Pennsylvania; and Departments of Medicine, Pathology and Surgery, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213.

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Address for reprint requests: Dr. Jessica H. Lewis, Central Blood Bank, 812 Fifth Avenue, Pittsburgh, Pennsylvania 15219.

TABLE 1. PATIENT SEX—RELATIONSHIP TO SHORT-TERM SURVIVAL AND HEPATITIS MARKERS*

		Markers‡					
Sex	*Survival†*	*None*	*Anti-A*	*Anti-B*	*Anti-C*	*Anti-D*	*HB_sAg*
F 267 (47)	226 (85)	78 (29)	153 (57)	53 (20)	29 (11)	3 (1)	26 (10)
M 306 (53)	244 (80)	80 (26)	176 (58)	51 (17)	71 (23)	18 (6)	70 (23)
573	470 (82)	158 (28)	329 (57)	104 (18)	100 (17)	21 (4)	96 (17)
P =	0.157	NS	NS	NS	<0.0002	0.005	<0.0001

*Values are numbers; percentages are in parentheses.
†Living at discharge from hospital admission, which includes primary liver transplant.
‡Percentages do not add up to 100 because one patient may have multiple markers.

Pittsburgh's IRB as exempt from the need for patient consent. When this research was started, the test for hepatitis C virus (HCV) was not yet approved by the FDA and all studies were nonlinked, ie, patient samples were coded and results were not reported to the patient or their physicians.

Samples were chosen randomly from those that appeared to have sufficient volume. The original plan was to choose about 600, roughly half of those available. The panel of diagnoses (see Tables 4 and 6) is not necessarily representative of that of all patients coming to OLTx over these years.

Clinicopathological Diagnoses. The diagnosis was made by clinical history, available serological tests, and examination of the extirpated native liver (1). Standard histopathological criteria were applied to the tissue sections (2).

Hepatitis markers. The following tests were carried out on each of 573 samples in Central Blood Bank's laboratories: anti-A—The RIA systems from Abbott Laboratories (Havab- and Havab-M-RIA); anti-B—the EIA system from Abbott Laboratories (Ausab EIA); anti-C—the EIA system from Chiron, distributed by Ortho Diagnostics (hepatitis C virus encoded antigen (recombinant C100-3) Ortho HCV ELISA test system); HB_sAg—the EIA system from Abbott Laboratories (Auszyme-Monoconal). Anti-delta or anti-D was tested only in the 185 samples that were positive for HB_sAg or for anti-B. The RIA system from Abbott Laboratories (Antidelta) was used.

Marker Patterns. To express the various combinations of antibodies and antigen found, patterns were constructed in which each antibody is expressed by its letter if reactive, by a dot (.) if nonreactive, or by a slash (/) if not tested. Antibodies are positioned in order—ABCD. These are followed by a hyphen (-) and then the mark for the antigen: B if HB_sAg is positive and dot if negative.

Statistical Methods. Statistical correlations and their significance were determined by the chi square test and/or Fisher's exact test.

RESULTS

Sex. Table 1 shows that there were 267 females (47%) and 306 males (53%) in this group of 573 primary liver transplant patients. Short-term survival is slightly better with females ($P = 0.157$). No markers were found in 29% of females and 26% of males. Anti-A and anti-B were about evenly distributed between the sexes. On the other hand, anti-C, anti-D and HB_sAg showed a striking difference, each being far less prevalent among females (all, $P < 0.0002$).

Year of Birth. Table 2 divides the patients by year of birth. Here significant trends are not readily detectable. Patients' ages do not relate to short-term survival or to the prevalence of markers with the exception of anti-A, which is more common in patients born before 1943 ($P < 0.001$).

Year of OLTx. Table 3 divides the patients by year of primary liver transplant. No significant trends are apparent.

TABLE 2. YEAR OF BIRTH—RELATIONSHIP TO SHORT-TERM SURVIVAL, HEPATITIS MARKERS, AND SEX*

			Markers*						Sex	
Birth year	N	*Survival**	*None*	*Anti-A†*	*Anti-B*	*Anti-C*	*Anti-D*	*HB_sAg*	*F*	*M*
08–22	19	19 (100)	3 (16)	15 (79)	6 (32)	4 (21)	1 (5)	3 (16)	10 (53)	9 (47)
23–32	120	91 (76)	29 (24)	77 (64)	22 (18)	14 (12)	2 (2)	21 (18)	49 (41)	71 (59)
33–42	160	137 (86)	37 (23)	100 (63)	21 (13)	33 (21)	4 (3)	25 (18)	81 (51)	79 (49)
43–52	163	133 (82)	57 (35)	80 (49)	30 (18)	30 (18)	8 (5)	29 (18)	67 (41)	96 (59)
53–62	71	55 (77)	21 (30)	34 (48)	17 (24)	13 (18)	4 (6)	13 (18)	37 (52)	34 (48)
63–71	40	35 (88)	11 (28)	23 (58)	8 (20)	6 (15)	2 (5)	5 (13)	23 (58)	17 (43)
Total	573	470 (82)	158 (28)	329 (57)	104 (18)	100 (17)	21 (4)	96 (17)	267 (47)	306 (53)

*See Table 1.
†Dividing birth year into two groups: 08–42 and 43–71, anti-A is significantly greater in the older group, $P = 0.0008$.

TABLE 3. YEAR OF LIVER TRANSPLANT—RELATIONSHIP TO SHORT-TERM SURVIVAL, HEPATITIS MARKERS, AND SEX*

OLTx year	N	Survival*	Markers*						Sex	
			None	Anti-A	Anti-B	Anti-C	Anti-D	HB_sAg	F	M
1982	5	3 (60)	0 (0)	3 (60)	1 (20)	2 (40)	1 (20)	3 (60)	2 (40)	3 (60)
1983	16	13 (82)	4 (25)	10 (63)	2 (13)	0 (0)	1 (6)	3 (19)	7 (44)	9 (56)
1984	30	23 (77)	13 (43)	13 (43)	5 (17)	2 (7)	3 (10)	7 (23)	20 (67)	10 (33)
1985	54	43 (80)	14 (26)	34 (63)	7 (13)	6 (11)	3 (6)	13 (24)	35 (65)	19 (35)
1986	69	60 (87)	23 (33)	37 (54)	11 (16)	13 (19)	2 (3)	10 (14)	36 (52)	33 (48)
1987	129	104 (81)	31 (24)	80 (62)	18 (14)	22 (17)	4 (3)	19 (15)	66 (51)	63 (49)
1988	190	157 (83)	46 (24)	113 (59)	39 (21)	39 (21)	5 (3)	31 (16)	71 (37)	119 (63)
1989	80	67 (84)	27 (34)	39 (49)	21 (26)	14 (18)	2 (3)	10 (13)	30 (38)	50 (62)
Total	573	470 (82)	158 (28)	329 (57)	104 (18)	100 (17)	21 (4)	96 (17)	267 (47)	306 (53)

*See Table 1.

Clinicopathological Diagnosis. Table 4 shows that short-term survival for the entire group was 82%. In Budd-Chiari, Wilson's disease, and hepatitis B, it was 60%, 67%, and 73%, respectively. Patients with no markers were most common in PSC ($P < 0.0001$), PBC ($P = 0.006$), and malignancy ($P = 0.001$). Anti-A was found in 57% of all patients. It was least frequent in PSC ($P = 0.0001$). Anti-B was found in 18% of all patients and varied from 10% in PBC to 32% in acute hepatitis. Anti-C was most frequent in NANB hepatitis (49%) but was also found in hepatitis B (35%) and cryptogenic cirrhosis (21%). HB_sAg was found in 96 patients (17%), the large majority of which were diagnosed as having chronic hepatitis B, Budd-Chiari, Wilson's disease, or acute hepatitis.

Table 4 also shows the sex distribution among the various diagnoses. Those suffering from PBC and from Budd-Chiari were 90% female. Hepatitis B (89%) and alcoholic cirrhosis (80%) occurred primarily in males. Sclerosing cholangitis, malignancy, α_1-antitrypsin deficiency, acute hepatitis, and cryptogenic cirrhosis all were found in 58% or more of males.

Interrelationship Between Anti-B, Anti-D, and HB_sAg and Short-Term Survival of Patients. HB_sAg and anti-B were tested on all patient samples (573), but anti-D was done only when either or both of these were positive (185 samples). Table 5 shows the results: 388 (68%) were negative for HB_sAg and anti-B. HB_sAg was positive in 96; alone in 66, with anti-D in 15, with anti-B in 10, and with both in 5. Anti-B was positive in 104; alone in 88, with anti-D in 1, and as stated in the last sentence, with HB_sAg in 10 and with both in 5. Short-term survival is shown in the last column. Anti-B tended to have a beneficial effect, short-term survival being 90% ($P = 0.08$ vs other patients). Patients with HB_sAg,

TABLE 4. CLINICOPATHOLOGICAL DIAGNOSES—RELATIONSHIP TO SHORT-TERM SURVIVAL, HEPATITIS MARKERS, AND SEX*

Diagnosis/ Disease†	N	Survival*	Markers*						Sex	
			None	Anti-A	Anti-B	Anti-C	Anti-D	HB_sAg	F	M
CC	100	85 (85)	20 (20)	61 (61)	24 (24)	21 (21)	0 (0)	0 (0)	42 (42)	58 (58)
PBC	99	85 (85)	41 (41)	50 (50)	9 (9)	4 (4)	0 (0)	8 (8)	90 (90)	9 (10)
PSC	81	66 (81)	40 (49)	30 (37)	14 (7)	6 (7)	0 (0)	2 (2)	30 (37)	51 (61)
ALC	56	46 (82)	15 (27)	36 (64)	13 (13)	7 (13)	3 (5)	8 (14)	11 (20)	45 (80)
HepB	55	40 (73)	0 (0)	41 (75)	8 (35)	19 (35)	14 (25)	47 (85)	6 (11)	49 (89)
MA	54	46 (85)	24 (44)	25 (46)	9 (11)	6 (11)	1 (2)	7 (13)	22 (41)	32 (59)
NANB	51	43 (84)	10 (20)	35 (69)	10 (49)	25 (49)	1 (2)	4 (8)	25 (49)	26 (51)
AcH	31	26 (84)	1 (3)	23 (74)	10 (19)	6 (19)	2 (6)	10 (32)	13 (42)	18 (58)
α_1AT	17	14 (82)	2 (12)	11 (59)	3 (18)	3 (18)	0 (0)	3 (18)	7 (41)	10 (59)
BC	10	6 (60)	1 (10)	6 (60)	1 (10)	1 (10)	0 (0)	4 (40)	9 (90)	1 (10)
WD	9	6 (67)	0 (0)	7 (78)	1 (11)	2 (22)	0 (0)	3 (33)	6 (66)	3 (33)
Misc‡	10	7 (70)	4 (40)	4 (40)	2 (20)	0 (0)	0 (0)	0 (0)	6 (60)	4 (40)
Total	573	470 (82)	158 (28)	329 (57)	104 (18)	100 (17)	21 (4)	96 (17)	267 (47)	306 (53)

*See Table 1.

†CC = cryptogenic cirrhosis; PBC = primary biliary cirrhosis, PSC = primary sclerosing cholangitis, ALC = alcoholic cirrhosis; HepB = hepatitis B, MA = malignancy; NANB = Non-A, non-B hepatitis; AcH = acute hepatitis; α_1AT = α_1-antitrypsin deficiency; BC = Budd-Chiari; WD = Wilson's disease.

‡Misc: uncertain diagnosis (8), posthepatic pediatric (1), hemachromatosis (1).

TABLE 5. NUMBERS AND SHORT-TERM SURVIVAL OF PATIENTS WITH HB_SAG AND/OR ANTI-B AND/OR ANTI-D

HB_SAg	Anti-B*	Anti-D	Number	Survival† No.	Survival† %
0	0	/	388	318	82
0	+	0	88	79	90
0	+	+	1	0	0
+	0	0	66	48	73
+	0	+	15	12	80
+	+	0	10	10	100
	+	+	5	3	60
			Total: 573	470	82
+/0	+/0		21	15	71

*With anti-B alone (P = 0.57) or in combination (P = 0.08) survival was improved.
†See Table 1.

alone or in combination with anti-D, had short-term survivals of 71–73%.

Hepatitis Marker Patterns. Table 6 shows, in the left-hand column, the 24 patterns that were found (28 patterns are possible; .B.D-., .BC.-B, AB.D-., ABCD-. were not exemplified). The short-term survival appears to relate to the marker pattern. In the lowest group in the left-hand column containing both anti-A and B antigen, the short-term survival is significantly lower (72%) than in the other three groups combined ($P < 0.02$), including B antigen without anti-A (86%) and anti-A without B antigen (83%).

Marker patterns that occurred in more than 20 patients are as follows: A../-.(180), .../-.(158), AB..-.(45), A ...-B(34), ..C/.(27), A.C/-.(23), ..C/-.(23) and .B..-.(22). Of these common patterns, reduced short-term survival was found only in A...-B.

DISCUSSION

The testing of hepatovirus markers is important in OLTx patients in two ways—in establishing a diagnosis and in demonstrating a risk factor. The high prevalence of anti-HAV (anti-A) in these patients raises the questions of whether this virus has

TABLE 6. MARKER PATTERNS—RELATIONSHIP TO SHORT-TERM SURVIVAL AND CLINICOPATHOLOGICAL DIAGNOSES

Marker	N	Living N	Living %	CC	PBC	PSC	ALC	HepB	MA	NANB	AcH	α_1AT	BC	WD	Misc
... /-.	158	129	82	20	41	40	15	0	24	10	1	2	1	0	4
.B..-.	22	21	95	6	3	7	0	1	3	0	1	0	0	0	1
..C/-.	27	23	85	11	1	4	1	1	0	3	1	3	0	1	1
.BC.-.	7	7	100	2	0	0	1	1	0	3	0	0	0	0	0
.BCD-.	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0
	215	180	84	39	45	51	18	3	27	16	3	5	1	1	6
....-B	19	16	84	0	4	0	0	6	1	0	3	1	3	1	0
...D-B	1	1	100	0	0	0	0	1	0	0	0	0	0	0	0
.B..-B	4	4	100	0	0	0	1	0	1	0	2	0	0	0	0
.B.D-B	1	1	100	0	0	0	1	0	0	0	0	0	0	0	0
..C.-B	1	1	100	0	0	0	0	1	0	0	0	0	0	0	0
..CD-B	2	1	50	0	0	0	0	2	0	0	0	0	0	0	0
.BCD-B	1	1	100	0	0	0	0	1	0	0	0	0	0	0	0
	29	25	86	0	4	0	2	11	2	0	5	1	3	1	0
A../-.	180	147	82	38	40	20	22	2	14	14	11	7	4	4	3
AB..-.	45	40	89	15	5	7	7	1	0	2	4	2	1	0	1
A.C/-.	23	19	83	7	1	1	1	0	3	10	1	0	0	0	0
ABC.-.	14	11	79	1	0	0	1	1	3	5	2	0	0	1	0
	262	217	83	61	46	28	31	4	20	31	18	9	5	5	4
A...-B	34	24	71	0	2	1	4	19	3	0	2	1	0	2	0
A..D-B	4	3	75	0	0	0	0	4	0	0	0	0	0	0	0
AB..-B	5	5	100	0	1	0	0	2	1	0	0	1	0	0	0
AB.D-B	2	1	50	0	0	0	0	0	1	0	1	0	0	0	0
A.C.-B	12	7	58	0	1	1	1	5	0	3	0	0	1	0	0
A.CD-B	8	7	88	0	0	0	0	6	0	1	1	0	0	0	0
ABC.-B	1	1	100	0	0	0	0	0	0	0	1	0	0	0	0
ABCD-B	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	67	48	72	0	4	2	5	37	5	4	5	2	1	2	0
Total	573	470	82	100	99	81	56	55	54	51	31	17	10	9	10

been underestimated as a cause of clinical disease or as a synergist for other hepatoviruses or as a predisposing factor for subsequent nonviral diseases. In other words, does a mild, limited hepatitis A infection leave enough liver damage to set the stage for additional liver disease? Only sclerosing cholangitis (PSC) shows no increase above the estimated US incidence rate of 35–40% in adults (3). Of particular interest is the recent description by Prochazka et al (4) of a strong genetic disposition in PSC. These authors demonstrated the HLA type DRW 52a in 100% of PSC patients tested. In this disease environmental factors such as virus exposure may not play any significant role. Of the patients with anti-A, only two were positive for IgM and both had been diagnosed with acute hepatitis A.

The hepatitis C virus (HCV) has been shown to be the major cause of NANB hepatitis (5). In our series, 25 (49%) of the 51 patients diagnosed as having NANB hepatitis showed the anti-C marker alone or in combination. Of the entire group of OLTx patients anti-C was found in 17%, a value similar to the 21% found in Italy (6).

The hepatitis D virus (delta agent) is an incomplete RNA virus that cannot enter mammalian cells without a hepatitis B DNA virus surface antigen coat (7). In this study anti-D was found in 20 of the 96 positive for HB_sAg (21%) and also in one patient with anti-B and no measurable HB_sAg in the circulation. This may have been a patient just converting from antigen positive to antibody positive.

Short-term survival used in many of these calculations is important only as it relates to perioperative deaths, rapid rejections, etc. Recurrence of disease, reinfection, rejection, etc, may not be evident at the time of hospital discharge. However, the best short-term prognosis for OLTx fits a female of any age with anti-B marker and the clinical diagnosis of primary biliary cirrhosis, cryptogenic cirrhosis, or malignancy.

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