Journal of Medical Virology 55:123–128 (1998)

Infection With GB Virus C, Hepatitis C and B Viruses in 1,044 Cases Autopsied at the Medical Examiner's Office in Tokyo

Jun Takamatsu,[1] Fumio Tsuda,[2] and Masahiko Okudaira[1]*

[1]Medical Examiner's Office, Tokyo Metropolitan Government, Tokyo 112, Japan
[2]Department of Medical Sciences, Toshiba General Hospital, Tokyo 140, Japan

Markers of GB virus C (GBV-C), hepatitis C virus (HCV) and hepatitis B virus (HBV) were sought in sera from 1,044 cases autopsied at the Medical Examiner's Office, Tokyo Metropolitan Government. GBV-C RNA was detected in 35 (3%) cases at a frequency significantly higher ($P < 0.05$) than in blood donors in Tokyo (4 of 448 or 1%). Three genotypes of GBV-C provisionally designated G1, G2 and G3 were determined by selective amplification with type-specific primers, and G3 (Asian type) was detected in 31 (89%), G2 (European/American type represented by the prototype hepatitis G virus) in three (9%) and G1 (West African type represented by the prototype GBV-C) in one (3%). Antibody to HCV (anti-HCV) was detected in 116 (11%) cases and accompanied by HCV RNA in 88. HCV genotypes were I/1a in one (1%), II/1b in 55 (63%), III/2a in 17 (19%) and IV/2b in 13 (15%). Antibodies to hepatitis B virus (HBV) was detected in 335 (32%) cases and hepatitis B surface antigen in 14 (1%). Subtypes were determined in 12 of them, *adw* was found in seven (58%), *adr* in four (33%) and *adyr* in one (8%). GBV-C RNA was detected significantly more frequently ($P < 0.01$) in the cases with liver disease (9 of 70 or 13%) than in those with the other causes of death (26 of 974 or 3%). Anti-HCV was more frequent in the cases with GBV-C RNA than in those without it (15 of 35 or 43% vs. 101 of 1,009 or 10%, $P < 0.001$). These results indicate that infection with GBV-C as well as HCV was common, while infection with HBV was not common in the Medical Examiner's autopsy cases in Tokyo. ***J. Med. Virol. 55:123–128, 1998.***

KEY WORDS: hepatitis viruses; hepatitis C viruses; hepatitis B virus; genotype; chronic liver disease; autopsy

INTRODUCTION

The discovery of GB virus C (GBV-C) [Simons et al., 1995; Leary et al., 1996] and hepatitis G virus [Linnen et al., 1996] has attracted considerable attention. These are separate isolates of the same virus in the *Flaviviridae* family, sharing >86% of nucleotides and >96% of deduced amino acid sequence, and referred to as GBV-C collectively for convenience. GBV-C is a well adapted human virus, and has penetrated into the general population. GBV-C RNA is found in 1~2% on the average and up to 14% of apparently healthy individuals in different countries [Dawson et al., 1996; Linnen et al., 1996; Masuko et al., 1996; Brown et al., 1997; Wang et al., 1997]. Hence, GBV-C is more prevalent than hepatitis C virus (HCV) or hepatitis B virus (HBV) on a worldwide scale.

GBV-C RNA is detected more often in the patients with acute or chronic non-A to E hepatitis than in normal controls represented by blood donors, and it is detected as frequently in the patients with HCV infection as in those with non-A to E liver diseases [Collombatto et al., 1996; Fiordalisi et al., 1996]. The possibility for GBV-C to induce posttransfusion hepatitis is ambiguous without any identifiable chronic sequelae [Wang et al., 1996; Alter et al., 1997]. Although GBV-C RNA is detected more often in patients with cryptogenic hepatitis/cirrhosis than in healthy blood donors, it is found as often or even more frequently in the hepatitis patients who are infected with HCV or HBV [Linnen et al., 1996].

GBV-C is transmitted efficiently by transfusions and along with illicit intravenous drugs [Aikawa et al., 1996; Masuko et al., 1996; Shimizu et al., 1996; Wang et al., 1996; Alter et al., 1997]. GBV-C infects babies perinatally with an efficiency lower than HBV but

Contract grant sponsor: Viral Hepatitis Research Foundation of Japan.

*Correspondence to: Masahiko Okudaira, Medical Examiner's Office, Tokyo Metropolitan Government, 4-21-18 Otsuka, Bunkyo-ku, Tokyo 112, Japan.

Accepted 3 December 1997

higher than HCV, particularly when mothers are co-infected with human immunodeficiency virus type 1 or HCV [Feucht et al., 1996; Viazov et al., 1997]. As is the case for HCV [Alter et al., 1990], however, a defined route of transmission is not identified in the majority of individuals with GBV-C RNA. Consequently, the exact mode of transmission to maintain a reservoir in the community is not clear, as yet.

GBV-C clusters in particular populations typified by hemodialysis patients, hemophiliacs and intravenous drug users [Aikawa et al., 1996; Jarvis et al., 1996; Linnen et al., 1996; Masuko et al., 1996; Tsuda et al., 1996; Kinoshita et al., 1997; Wang et al., 1997]. We surveyed GBV-C RNA and markers of HCV and HBV infection in 1,044 cases autopsied at the Medical Examiner's Office, Tokyo Metropolitan Government. Infections with GBV-C and HCV were found to be more prevalent as compared to HBV.

MATERIALS AND METHODS

Studied Subjects

During December, 1993 through September, 1994 at the Medical Examiner's Office, Tokyo Metropolitan Government, 2,104 cases were necropsied. Among these, 1,044 cases of the Japanese nationality were autopsied within 48 hr after the estimated time of death (mean 19 ± 11 hr). They included 779 men (mean ± SD age: 55 ± 16 years [range: 0–95 years]) and 265 women (62 ± 18 years [range: 0–96 years]). The majority were sudden and unexpected deaths and including accidental and suicidal deaths. Heart blood was obtained at autopsy, and sera was separated and stored at −80°C until tested for markers of GBV-C, HCV and HBV infections.

Detection of GBV-C RNA

GBV-C RNA was determined by reverse-transcription (RT)—polymerase chain reaction (PCR) with primers deduced from the 5′ untranslated region (UTR) of the GBV-C genome by the method described previously [Shimizu et al., 1996].

GBV-C Genotypes

Three genotypes of GBV-C, provisionally designated G1, G2 and G3 [Okamoto et al., 1997], were determined by selective amplification with type-specific primers deduced from the 5′UTR [Fukushi et al., 1996; Leary et al., 1996; Linnen et al., 1996; Muerhoff et al., 1996; Shao et al., 1996; Okamoto et al., 1997] by the method described previously [Shrestha et al., 1997].

Markers of the Other Viruses

Antibody to HCV (anti-HCV) was detected by a second-generation enzyme-linked immunosorbent assay (ELISA) with commercial kits (HCV ELISA II, Ortho Diagnostic Systems, Tokyo, Japan). Samples with absorbance at 492 nm > 0.65 were considered reactive, and they were tested for HCV RNA by PCR with nested primers deduced from the 5′UTR [Okamoto et al., 1994]. The five common HCV genotypes designated by the mixed nomenclature (I/1a, II/1b, III/2a, IV/2b and V/3a) were determined by selective amplification by type-specific primers deduced from the core gene [Okamoto, 1996].

HBsAg and the corresponding antibody (anti-HBs) were determined by passive hemagglutination with commercial kits (MyCell II HBsAg and anti-HBs, Institute of Immunology Co., Ltd., Tokyo, Japan), and samples with hemagglutination titers $\geqslant 2^2$ scored positive. Two sets of allelic subtypic determinants of HBsAg, *d/y* and *w/r,* were determined by ELISA with commercial kits (HBsAg SUBTYPE EIA, Institute of Immunology Co., Ltd.) and another set, *i/t,* by ELISA with monoclonal antibodies [Ohnuma et al., 1993]. Hepatitis B *e* antigen (HBeAg) and the corresponding antibody (anti-HBe) were determined in sera positive for HBsAg by commercial kits (IMMUNIS HBeAg/HBeAb EIA, Institute of Immunology Co., Ltd.). Antibody to hepatitis B core (anti-HBc) was determined by hemagglutination inhibition by the method described elsewhere [Iizuka et al., 1992], and samples with titers $\geqslant 2^3$ were considered positive. Sera positive for anti-HBs, anti-HBc or both were conceived to contain antibody to HBV (anti-HBV).

Statistical Analyses

The frequency between groups was compared using χ^2 test, and Fisher's exact test.

RESULTS

Infection with GBV-C, HCV and HBV among 1,044 Autopsied Cases

GBV-C RNA was detected in 35 of 1,044 (3%) cases, at a frequency significantly higher ($P < 0.05$) than in four of the 448 (1%) blood donors without anti-HCV, HBsAg or elevated alanine aminotransferase (ALT) levels ⩽45 IU/L). Anti-HCV was detected in 116 (11%) cases, more frequently ($P < 0.001$) than in the blood donors matched for the age (11 of 923 or 1%) [Fukuda et al., 1994], and 88 (76%) were positive for HCV RNA. HBsAg was detected in 14 (1%) cases, at a frequency comparable with the age-matched blood donors in Japan surveyed before the screening for HBsAg [Tanaka et al., 1986]. HBeAg was detected in two (14%) and the other 11 were positive for anti-HBe; neither HBeAg nor anti-HBe was detected in the remaining one.

The age-specific prevalence of GBV-C RNA as well as markers of HCV and HBV infections are illustrated in Fig. 1. GBV-C RNA was not detected in any of the 67 cases younger than 30, while it was found in 34 of the 734 (5%) cases aged from 30 to 69. The prevalence of anti-HCV also increased with age, and positive in one (1%) of the cases under 30 years but in 4–16% of those in older age groups. Likewise, anti-HBV increased with age; it was detected in 10% of the age group less than 30 years while in 25–41% of older age groups.

Of the 35 cases with GBV-C RNA, 15 (43%) were positive for anti-HCV of whom 14 (93%) had HCV RNA. Anti-HCV was detected in 101 of the 1,009 (10%) cases without GBV-C RNA, by contrast, and HCV RNA was detected in 74 (73%) of them. Thus anti-HCV was more

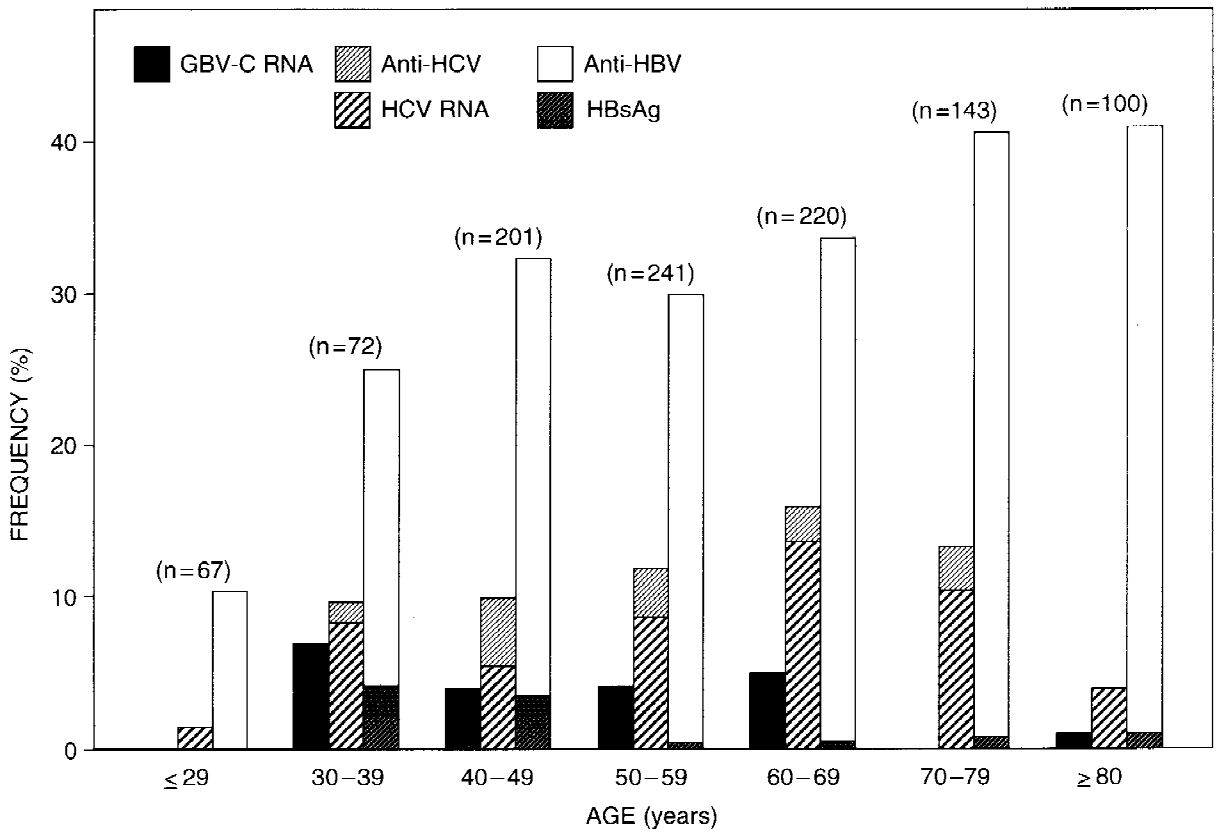

Fig. 1. Age-specific prevalence rates of GBV-C RNA as well as markers of HCV and HBV infections in 1,044 cases. HCV RNA was tested only in sera positive for anti-HCV. Anti-HBV denotes antibody to HBsAg, antibody to HBV core or both. All sera with HBsAg contained antibody to HBV core.

frequent in the cases with GBV-C RNA than those without it ($P < 0.001$). None of the 35 cases with GBV-C RNA possessed HBsAg, while it was detected in 14 of the 1,009 (1%) cases without GBV-C RNA.

Genotypes of GBV-C and HCV as Well as HBsAg Subtypes

GBV-C genotypes have been classified into G1, G2 and G3 based on the sequence divergence within the entire genome [Okamoto et al., 1997] or a part of 5′UTR [Muerhoff et al., 1996]. G3 was detected in 31 of the 35 (89%) cases with GBV-C RNA; this genotype is most prevalent in Japan and detected in 24 of 25 (96%) symptom-free carriers [Okamoto et al., 1997]. G2 was found in three (9%); it is an American type represented by the prototype hepatitis G virus [Linnen et al., 1996]. Only one (3%) was classified as G1, the genotype of the prototype GBV-C and prevalent in West Africa [Simons et al., 1995].

HCV genotypes in the 88 cases with the viral RNA were classified into I/1a in one (1%), II/1b in 55 (63%), III/2a in 17 (19%), and IV/2b in 13 (15%); genotypes were not classifiable into the common five for the remaining two (2%) cases due to low HCV RNA titers. The distribution of HCV genotypes was comparable with that in symptom-free carriers and patients with chronic liver disease in Japan [Okamoto and Mishiro, 1994]. There were no differences in the distribution of HCV genotypes in the cases with and without GBV-C RNA.

Of the 14 samples with HBsAg, titers were too low to allow subtyping in two. HBsAg subtypes in the remaining 12 samples were *adw* in seven (58%), *adr* in four (33%) and *adyr* in one (8%). The distribution of *adw* and *adr* subtypes was somewhat different from that in the inhabitants of Tokyo in a previous study where HBsAg/*adw* was detected in 33% and HBsAg/*adr* in 67% [Yamashita et al., 1975]. Of the seven cases with HBsAg/*adw,* six were under 45 years of age, and the remaining one was 68 years old. The four cases with HBsAg/*adr* were aged from 43 to 52 years. Of the subtypic determinants of HBsAg in a third allele [Ohnuma et al., 1993], *i* was detected in all HBsAg/*adr* samples while *t* was found in every HBsAg/*adw* sample. The association of *i* with *adr* and that of *t* with *adw* have been reported in Japanese HBsAg carriers [Ohnuma et al., 1993].

Markers of GBV-C, HCV and HBV in Cases with Different Causes of Death

Table I compares prevalence rates of GBV-C RNA, as well as markers of HCV and HBV infections, in cases classified by the cause of death determined by autopsy procedure. A cardiovascular disease was most frequent

TABLE I. Markers of GBV-C, HCV and HBV Infections in Cases of Death by Various Causes

Cause of death	*n*	Male (%)	GBV-C RNA	Anti-HCV	HCV RNA[a]	HBsAg	Anti-HBV[b]
Cardiovascular	456	346 (76%)	10 (2%)	37 (8%)	24 (5%)	5 (1%)	142 (31%)
Respiratory	138	96 (70%)	3 (2%)	12 (9%)	9 (7%)	2 (1%)	45 (33%)
Cerebrovascular	75	47 (63%)	2 (3%)	12 (16%)	9 (12%)	0	25 (33%)
Hepatic	70	61 (87%)	9 (13%)	24 (34%)	18 (26%)	4 (6%)	23 (33%)
Trauma	59	50 (85%)	5 (9%)	3 (5%)	3 (5%)	0	26 (44%)
Drowned	50	38 (76%)	0	4 (8%)	4 (8%)	1 (2%)	14 (24%)
Cold exposure	46	42 (91%)	1 (2%)	10 (22%)	10 (22%)	0	21 (46%)
Gastrointestinal	44	33 (75%)	1 (2%)	7 (16%)	5 (11%)	1 (2%)	8 (18%)
Toxic	31	35 (81%)	1 (3%)	5 (16%)	7 (13%)	0	11 (35%)
Others	75	41 (55%)	3 (4%)	2 (3%)	2 (3%)	1 (1%)	20 (27%)
Total	1044	779 (75%)	35 (3%)	116 (11%)	88 (8%)	14 (1%)	335 (32%)

[a]Tested only in sera positive for anti-HCV.
[b]Positive for anti-HBs, anti-HBc or both.

and diagnosed in 456 (44%) cases and followed by respiratory and cerebrovascular diseases in 138 (13%) and 75 (7%) cases, respectively. A hepatic disease was found in 70 (7%) cases. GBV-C RNA was detected in nine (13%) of them at a frequency significantly higher ($P < 0.01$) than in 26 of the other 974 (3%) cases with non-hepatic diseases. Also, anti-HCV was detected more frequently in the cases with liver disease than in those with the other diseases (24 of 70 or 34% vs. 92 of 974 or 9%, $P < 0.001$).

Anti-HCV was detected in 10 of the 46 (22%) cases with death due to cold exposure, at a frequency significantly higher ($P < 0.01$) than that in 82 of the 928 (9%) cases with the other non-hepatic diseases. HCV RNA was detected in all the ten cases with anti-HCV who died due to cold exposure; it was detected only in 78 of 106 (74%) cases with anti-HCV having the other causes of death.

Markers of GBV-C, HCV and HBV Infections in the 70 Cases with Liver Disease

Table II compares markers of GBV-C, HCV and HBV infections in the 70 cases with liver diseases classified by etiology. GBV-C RNA was detected in nine, of whom six had HCV RNA also. Anti-HCV was found in 24 and HCV RNA was detected in 18 of the 70 cases. Neither GBV-C RNA nor anti-HCV was detectable in 46, and 17 of these had anti-HBs including the four with HBsAg.

Alcoholic liver diseases, eventualy in most of which were alcoholic fatty liver and cirrhosis, were most prevalent and found in 46 cases (66%). Anti-HCV was detected in 17 (37%), 11 of whom had HCV RNA. Therefore, viral hepatitis was not excluded in some cases with alcoholic liver disease. Twelve cases of posthepatitic type of liver cirrhosis without liver cancer were found.

All seven cases with hepatocellular carcinoma were accompanied by posthepatitic type of cirrhosis, and five had HCV RNA and two HBsAg without anti-HCV. Remarkably, three with HCV RNA were included in the six cases with both HCV RNA and GBV-C RNA. The other three cases with both GBV-C RNA and HCV RNA had alcoholic liver disease.

DISCUSSION

The Medical Examiner's Office, Tokyo Metropolitan Government examines all deaths due to infectious disease, intoxication, accident, suicide, and unknown etiology in the Tokyo urban area in accordance with "the dead body autopsy and preservation law" of Japan. Consequently, when the cause of death cannot be determined by the Medical Examiner's external inspection of the body and data investigated by the Metropolitan Police Department, an autopsy examination is mandatory in order to identify the cause of death. Deaths due to homicide are excluded, because it is investigated by the Department of Forensic Medicine in Universities in Tokyo.

A total of 1,044 cases were autopsied within 48 hours after death during 10 months from December 1993 to September, 1994 in the Medical Examiner's Office, Tokyo Metropolitan Government. The majority were cases with so-called sudden and unexpected death and unknown causes of death, and were found dead on arrival at hospital or died on the street. Hence, they were heavily biased for a population with a low socioeconomic status in Tokyo.

GBV-C RNA was detected in 35 of the 1,044 (3%) cases at a frequency significantly higher than in four of the 448 (1%) of blood donors in Tokyo ($P < 0.05$). Anti-HCV also was frequent and positive in 116 (11%) cases, and was accompanied by HCV RNA in 88. The frequency of anti-HCV in these cases was higher than in the age-matched control (11 of 923 or 1%) [Fukuda et al., 1994]. HBsAg was detected in only 14 (1%) cases, in contrast to 2% in Japanese blood donors in an area before the screening for HBsAg [Tanaka et al., 1986]. Anti-HCV was found significantly more often in the cases with GBV-C RNA than in those without it (15 of 35 or 43% vs. 101 of 1,009 or 10%, $P < 0.001$), while HBsAg was not accompanied by GBV-C RNA.

These results highlight high prevalence rates of GBV-C and HCV as well as co-infection with both in cases investigated in Tokyo. It is of note that the frequency of GBV-C RNA (3%) was lower than that of HCV RNA (at least 88 in 1,044 or 8%). Since GBV-C infection by transfusion becomes persistent only in

TABLE II. Markers of GBV-C, HCV and HBV Infections in Cases of Death by Various Liver Diseases

Disease	*n*	Male (%)	GBV-C RNA	Anti-HCV	HCV RNA	HBsAg	Anti-HBV
Alcoholic	46	40 (87%)	6 (13%)	17 (37%)	11 (24%)	1 (2%)	14 (30%)
Cirrhosis	12	11 (92%)	0	2 (17%)	2 (17%)	0	5 (42%)
Carcinoma	7	6 (86%)	3 (43%)	5 (71%)	5 (71%)	2 (29%)	2 (29%)
Others[a]	5	4 (80%)	0	0	0	1 (20%)	2 (40%)
Total	70	61 (87%)	9 (13%)	24 (34%)	18 (26%)	4 (6%)	23 (33%)

[a]Included one body with hepatitis B, two with Reye's syndrome, one with nonspecific hepatitis and one with cholangitis.

36% of recipients [Pilot-Matias et al., 1996; Wang et al., 1996], much less often than HCV infection which leads to chronicity in up to 80%, the cases exposed to GBV-C would have been much higher than 3% estimated by the detection of viral RNA. Tests for antibody to the E2 protein of GBV-C, which is detectable in the individuals with resolved infection [Pilot-Matias et al., 1996; Dille et al., 1997; Tacke et al., 1997], would be required to estimate an actual exposure rate to GBV-C.

Three GBV-C genotypes are distinguished by sequence divergence >12% within the entire genome or subgenomic regions [Muerhoff et al., 1996; Okamoto et al., 1997], and they are provisionally designated G1 (West African type), G2 (American type) and G3 (Asian type) [Simons et al., 1995; Linnen et al., 1996; Okamoto et al., 1997]. GBV-C genotypes were determined by PCR with type-specific primers deduced from the 5′UTR [Shrestha et al., 1997]. The distribution of the three GBV-C genotypes in the 35 cases with the viral RNA was no different from that in blood donors in Japan, with G3 most prevalent and found in 31 (89%). Hence, GBV-C in them would hardly have a foreign origin as was identified in the Japanese patients with hemophilia who had received inadequately sterilized blood products; GBV-C of genotype 1 occurred in ten of the 19 (53%) infected hemophiliacs (unpublished observations). This view was supported by the distribution of HCV genotypes in the cases with HCV RNA, which was comparable with that in HCV carriers identified in blood donors and patients with chronic liver disease in Japan [Okamoto and Mishiro, 1994].

There are two subtypes of HBsAg in Japan, i.e., *adw* and *adr.* Within mainland Japan, there is a south-to-north gradient from *adr* to *adw,* which is believed to represent the migration of Japanese ancestors who immigrated from the Korean peninsula and migrated toward the north [Yamashita et al., 1975]. The distribution of *adw* to *adr* in the cases with HBsAg antigenemia was 7 : 4, which seems to be at variance with 3 : 7 in the general population in Japan represented by blood donors [Yamashita et al., 1975]. The cases with HBsAg/*adw* were younger than those with HBsAg/*adr,* which might represent a particular route of transmission such as intravenous drug abuse.

Co-infection with GBV-C and HCV was frequent, which reinforces a common route of transmission of the two viruses reported previously [Aikawa et al., 1996; Linnen et al., 1996; Masuko et al., 1996]. Moreover, GBV-C RNA was detected in three of the five (60%) cases with hepatocellular carcinoma with anti-HCV and HCV RNA. This may corroborate the report of Berg et al. [1996] who found primary hepatocellular carcinoma in five of the six (83%) recipients of liver transplantation co-infected with GBV-C and HCV more frequently ($P < 0.01$) than in 16 of the 68 (23%) with HCV infection alone.

ACKNOWLEDGMENTS

The authors thank Professor Gotaro Toda, the Jikei University School of Medicine, Drs. Munesuke Shoji (Chief Medical Examiner), Shogo Tokudome (Deputy Chief Medical Examiner) and all the staff members of the Medical Examiner's Office, Tokyo Metropolitan Government as well as Professor Makoto Mayumi, Immunology Division, Jichi Medical School, Japan for valuable help throughout this work.

REFERENCES

Aikawa T, Sugai Y, Okamoto H (1996): Hepatitis G infection in drug abusers with chronic hepatitis C [letter]. New England Journal of Medicine 334:195–196.

Alter HJ, Nakatsuji Y, Melpolder J, Wages J, Wesley R, Shih JW, Kim JP (1997): The incidence of transfusion-associated hepatitis G virus infection and its relation to liver disease. New England Journal of Medicine 336:747–754.

Alter MJ, Hadler SC, Judson FN, Mares A, Alexander WJ, Hu PY, Miller JK, Moyer LA, Fields HA, Bradley DW, Margolis HS (1990): Risk factors for acute non-A, non-B hepatitis in the United States and association with hepatitis C virus infection. Journal of the American Medical Association 264:2231–2235.

Berg T, Naumann U, Fukumoto T, Bechstein WO, Neuhaus P, Lobeck H, Hohne M, Schreier E, Hopf U (1996): GB virus C infection in patients with chronic hepatitis B and C before and after liver transplantation. Transplantation 62:711–714.

Brown KE, Wong S, Buu M, Binh TV, Be TV, Young NS (1997): High prevalence of GB virus C/hepatitis G virus in healthy persons in Ho Chi Minh City, Vietnam. Journal of Infectious Diseases 175: 450–453.

Collombatto P, Randone A, Civitico G, Monti Gorin J, Doki L, Medaina N, Oliveri F, Verme G, Marchiaro G, Pange R, Karayiannis P, Thomas C, Heso G, Bonino F, Brunetto MR (1996): Hepatitis G virus RNA in the serum of patients with elevated gamma glutamyl transpeptidase and alkaline phosphatase: a speific liver disease. Journal of Viral Hepatitis 3:301–306.

Dawson GJ, Schlauder GG, Pilot-Matias TJ, Thiele D, Leary TP, Murphy P, Rosenblatt JE, Simons JN, Martinson FE, Gutierrez RA, Lentino JR, Pachucki C, Muerhoff AS, Widell A, Tegtmeier G, Desai S, Mushahwar IK (1996): Prevalence studies of GB virus-C infection using reverse transcriptase-polymerase chain reaction. Journal of Medical Virology 50:97–103.

Dille BJ, Surowy TK, Gutierrez RA, Coleman PF, Knigge MF, Carrick RJ, Aach RD, Hollinger B, Stevens CE, Barbosa LH, Nemo GJ, Mosley JW, Dawson GJ, Mushahwar IK (1997): An ELISA for detection of antiboies to E2 protein of GB virus C. Journal of Infectious Diseases 175:458–461.

Feucht HH, Zollner B, Polywka S, Laufs R (1996): Vertical transmission of hepatitis G [letter]. Lancet 347:615–616.

Fiordalisi G, Zanella I, Mantero G, Bettinardi A, Stellini R, Paraninfo G, Cadeo G, Primi D (1996): High prevalence of GB virus C infec-

tion in a group of Italian patients with hepatitis of unknown etiology. Journal of Infectious Diseases 174:181–183.

Fukuda S, Suzuki T, Nagayama R, Tsuda F, Kojima M, Okamoto H, Tanaka T, Miyakawa Y, Mayumi M (1994): Hepatitis C virus RNA in blood units with antibodies detectable by a second-generation passive hemagglutination assay, antibodies to synthetic core peptides or elevated transaminase levels. Transfusion Science 15:83–92.

Fukushi S, Kurihara C, Ishiyama N, Okamura H, Hoshino FB, Oya A, Katayama K (1996): Nucleotide sequence of the 5′ noncoding region of hepatitis G virus isolated from Japanese patients: comparison with reported isolates. Biochemical and Biophysical Research Communications 226:314–318.

Iizuka H, Ohmura K, Ishijima A, Satoh K, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M (1992): Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. Vox Sanguinis 63:107–111.

Jarvis LM, Davidson F, Hanley JP, Yap PL, Ludlam CA, Simmonds P (1996): Infection with hepatitis G virus among recipients of plasma products. Lancet 348:1352–1355.

Kinoshita T, Miyake K, Nakao H, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M (1997): Molecular investigation of GB virus C infection in hemophiliacs in Japan. Journal of Infectious Diseases 175:454–457.

Leary TP, Muerhoff AS, Simons JN, Pilot-Matias TJ, Erker JC, Chalmers ML, Schlauder GG, Dawson GJ, Desai SM, Mushahwar IK (1996): Sequence and genomic organization of GBV-C: A novel member of the *Flaviviridae* associated with human non-A-E hepatitis. Journal of Medical Virology 48:60–67.

Linnen J, Wages J, Zhang-Keck ZY, Fry KE, Krawczynski KZ, Alter H, Koonin E, Gallagher M, Alter M, Hadziyannis S, Karayiannis P, Fung K, Nakatsuji Y, Shih JWK, Young L, Piatak M, Hoover C, Fernandez J, Chen S, Zou JC, Morris T, Hyams KC, Ismay S, Lifson JD, Hess G, Foung SKH, Thomas H, Bradley D, Margolis H, Kim JP (1996): Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science 271:505–508.

Masuko K, Mitsui T, Iwano K, Yamazaki C, Okuda K, Meguro T, Murayama N, Inoue T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M (1996): Infection with hepatitis GB virus C in patients on maintenance hemodialysis. New England Journal of Medicine 334:1485–1490.

Muerhoff AS, Simons JN, Leary TP, Erker JC, Chalmers ML, Pilot-Matias TJ, Dawson GJ, Desai SM, Mushahwar IK (1996): Sequence heterogeneity within the 5′-terminal region of the hepatitis GB virus C genome and evidence for genotypes. Journal of Hepatology 25:379–384.

Ohnuma H, Machida A, Okamoto H, Tsuda F, Sakamoto M, Tanaka T, Miyakawa Y, Mayumi M (1993): Allelic subtypic determinants of hepatitis B surface antigen (i and t) that are distinct from d/y or w/r. Journal of Virology 67:927–932.

Okamoto H, Mishiro S (1994): Genetic heterogeneity of hepatitis C virus. Intervirology 37:68–76.

Okamoto H, Mishiro S, Tokita H, Tsuda F, Miyakawa Y, Mayumi M (1994): Superinfection of chimpanzees carrying hepatitis C virus of genotype II/1b with that of genotype III/2a or I/1a. Hepatology 20:1131–1136.

Okamoto H, Kobata S, Tokita H, Inoue T, Woodfield GD, Holland PV, Al-Knawy BA, Uzunalimoglu O, Miyakawa Y, Mayumi M (1996): A second-generation method of genotyping hepatitis C virus by the polymerase chain reaction with sense and antisense primers deduced from the core gene. Journal of Virological Methods 57:31–45.

Okamoto H, Nakao H, Inoue T, Fukuda M, Kishimoto J, Iizuka H, Tsuda F, Miyakawa Y, Mayumi M (1997): The entire nucleotide sequences of two GB virus C/hepatitis G virus isolates of distinct genotypes from Japan. Journal of General Virology 78:737–745.

Pilot-Matias TJ, Carrick RJ, Coleman PF, Leary TP, Surowy TK, Simons JN, Muerhoff AS, Buijk SL, Chalmers ML, Dawson GJ, Desai SM, Mushahwar IK (1996): Expression of the GB virus C E_2 glycoprotein using the Semliki Forest virus vector system and its utility as a serologic marker. Virology 225:282–292.

Shao L, Shinzawa H, Ishikawa K, Zhang X, Ishibashi M, Misawa H, Yamada N, Togashi H, Takahashi T (1996): Sequence of hepatitis G virus genome isolated from a Japanese patient with non-A-E-hepatitis: amplification and cloning by long reverse transcription-PCR. Biochemical and Biophysical Research Communications 228:785–791.

Shrestha SM, Shrestha S, Tsuda F, Sawada N, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M (1997): Infection with GB virus C and hepatitis C virus in drug addicts, patients on maintenance hemodialysis, or with chronic liver disease in Nepal. Journal of Medical Virology, 53:157–161.

Shimizu M, Osada K, Okamoto H (1996): Transfusion-transmitted hepatitis G virus following open heart surgery [letter]. Transfusion 36:937.

Simons JN, Leary TP, Dawson GJ, Pilot-Matias TJ, Muerhoff AS, Schlauder GG, Desai SM, Mushahwar IK (1995): Isolation of novel virus-like sequences associated with human hepatitis. Nature Medicine 1:564–569.

Tacke M, Kiyosawa K, Stark K, Schlueter V, Ofenloch-Haehnle B, Hess G, Engel AM (1997): Detection of antibodies to a putative hepatitis G virus envelope protein. Lancet 349:318–320.

Tanaka T, Nagai M, Yoshihara S, Imai S, Ishiguro H, Seto S, Tsukada T, Tsuda F, Miyakawa Y, Mayumi M (1986): Changing pattern of age-specific prevalence of hepatitis B surface antigen and corresponding antibody in Japan. American Journal of Epidemiology 124:368–371.

Tsuda F, Hadiwandowo S, Sawada N, Fukuda M, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M (1996): Infection with GB virus C (GBV-C) in patients with chronic liver disease or on maintenance hemodialysis in Indonesia. Journal of Medical Virology 49:248–252.

Viazov S, Riffelmann M, Sarr S, Ballauff A, Meisel H, Roggendorf M (1997): Tranasmission of GBV-C/HBV from drug-addicted motehrs to their babies. Journal of Hepatology 27:85–90.

Wang JT, Tsai FC, Lee CZ, Chen PJ, Sheu JC, Wang TH, Chen DS (1996): A prospective study of transfusion-transmitted GB virus C infection: similar frequency but different clinical presentation compared with hepatitis C virus. Blood 88:1881–1886.

Wang Y, Chen HS, Fan MH, Liu HL, An P, Sawada N, Tanaka T, Tsuda F, Okamoto H (1997): Infection with GB virus C and hepatitis C virus in hemodialysis patients and blood donors in Beijing. Journal of Medical Virology 52:26–30.

Yamashita Y, Kurashina S, Miyakawa Y, Mayumi M (1975): South-to-north gradient in distribution of the r determinant of hepatitis B surface antigen in Japan. Journal of Infectious Diseases 131: 567–569.