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Original Article

Prevalence and Genotypic Distribution of Hepatitis GB-C/HG and TT Viruses in Blood Donors, Mentally Retarded Children and Four Groups of Patients in Eastern Anatolia, Turkey

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SUMMARY: We investigated the prevalence and genotypic distribution of GB virus-C/hepatitis G virus (GBV-C/HGV) and TT virus (TTV) in blood donors, mentally retarded children and four groups of patients living in Eastern Anatolia, Turkey. The prevalence and genetic analysis of TTV were determined by using the primers of the UTR and ORF1 regions of TTV, respectively. Reverse transcription nested (RT-n)-PCR was used to amplify 5´ UTR of GBV-C/HGV. Genotyping of HGV was carried out by PCR-based genotyping assay while RFLP was conducted to determine the genotypes of TTV. TTV DNA was detected in 118 of 410 sera tested, giving an overall prevalence of 28.7%; GBV-C/HGV-RNA was detected in only 17 cases, giving an overall prevalence of 4.1%. No significant differences were observed in the number of positive or negative tests for GBV-C/HGV and TTV according to duration of illness or mean duration of institutionalization in any of the groups studied. Although all samples from the study population belonged to genotypes 1 and 4, the most common TTV genotype is G2. In conclusion, our results indicate a low endemicity of GBV-C/HGV and TTV infection in Eastern Anatolia, Turkey. The presence of G2 strains reveals the limited genetic diversity of the GBV-C/HGV circulating in Turkey. We suggest that TTV infection of genotypes 1 and 4 is prevalent in the same region.

INTRODUCTION

GB virus-C/hepatitis G virus (GBV-C/HGV) is an envelope positive-stranded RNA virus with a genome of about 9.4 kb belonging to the *Flaviviridae* family. It is distributed worldwide, and at least five major genotypes of this virus have been proposed based on sequence analysis of the 5´ non-coding region 5´ (NCR) or E2 gene (1-3). GBV-C/HGV is transmitted through blood transfusion and blood components (4). Epidemiological data suggest that this virus can also spread by sexual or vertical transmission (5,6), however, little is known about other modes of transmission that could explain its high prevalence and worldwide distribution. Different modes of transmission have been proposed to explain the ubiquitous presence of this virus; for example, a high prevalence (22 to 38%) of viral RNA has been reported in populations with parenteral risk (7,8). However, similar prevalence rates have also been observed in groups with little or no parenteral risk, such as blood donors (7-9).

The TT virus (TTV) is a non-enveloped virus with a single-stranded circular DNA genome of approximately 3.8 kb (10). The significant diversity of the TTV genome allows for its classification into more than 30 genotypes at present (11,12). Several studies have shown that TTV is distributed widely throughout the world (13,14). Furthermore, undetected variants of TTV may also exist in unexamined countries. This virus may circulate among people by fecal-oral transmission, and the environmental or lifestyle risks of acquiring TTV infection from feces may differ by geographic region. It is known that TTV differs from other enterically transmitted viruses in that it can establish persistent infection (15), which facilitates its transmission through parenteral and nonparenteral routes in the general population. It has also been shown that individuals at high risk of contracting parenterally transmitted viruses, such as hemophiliacs, hemodialysis patients and intravenous drug users, are particularly susceptible to this infection (16).

Mentally retarded children, schizophrenic and leprosy patients can be expected to behave in an uncontrolled fashion with respect to personal hygiene. Therefore, the duration of institutionalization, the manner of using the living environment, and personal hygiene and behavioral factors such as nail-biting, sucking fingers and the frequency of daily hand washes may be factors that affect the prevalence of TTV and GBV-C/HGV infections in these groups. In addition, such patients may be at high risk of acquiring TTV and GBV-C/HGV since they undergo medical procedures related to bleeding at their hospital of institutionalization.

To the best of our knowledge, the estimated prevalences of TTV and GBV-C/HGV infections in Turkey have not been well-documented. Little is known about the genotype distribution of GBV-C/HGV and TTV strains circulating in this country. Therefore, the present study was undertaken to elucidate the prevalence and genotype distribution of GBV-C/HGV and TTV in blood donors, mentally retarded children, schizophrenic patients, leprosy patients, and chronic hepatitis B and C patients living in the Eastern Anatolia region of Turkey.

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MATERIALS AND METHODS

Study population: This study was conducted between September and December of 2003. A total of 410 patients were included in the study: 125 blood donors, 88 mentally retarded children, 56 schizophrenic patients, 43 leprosy cases, 51 chronic hepatitis B patients, and 47 chronic hepatitis C patients. This study was performed according to the protocol approved by the local Ethics Committee at the Firat University Medical School and was approved by the authorities of Elazig Psychiatric Hospital and Elazig Leprosy Hospital.

Blood donors: The blood donor group consisted of 125 healthy volunteers (94 males, 31 females; age range between 15 and 70 [35.01 ± 10.96]). For the blood donors, inclusion criteria were negative results for all hepatitis virus serologic markers, the absence of any acute or chronic infection, and being anti-HIV negative.

Mentally retarded children: All of the mentally retarded children included in the study were attending public schools for special education in the Elazig, Malatya and Diyarbakir Provinces in the Eastern Anatolia region of Turkey. Eighty-eight students (60 boys, 28 girls; age range between 6 and 25 [14.3 ± 4.5]) out of a total of 112 (78.5%) participated in the study. These schools are educational institutions that offer 5 to 7 h of education per day and that accept students between 7 and 25 years of age and who have been defined by a psychiatry clinic, pediatric psychiatry clinic or pediatric clinic to have educable mental retardation (IQ > 25). Information about the study and consent forms were prepared and provided to the parents of the subjects. Thus, the parents were informed, and their consent was obtained. In addition, a survey form was filled out for each participant with the help of the subject, his or her parents, and school records.

Schizophrenic patients: Fifty-six schizophrenic patients (32 males, 24 females; age range between 17 and 73 [43.8 ± 9.6]) who had been diagnosed according to Diagnostic and Statistical Manual of Mental Disorders-IV criteria and were hospitalized at the Elazig Psychiatric Hospital were included in this study. The Elazig Psychiatric Hospital, where this study was performed, is one of the biggest and oldest mental hospitals in Turkey, and is a regional hospital of Eastern Anatolia serving 18 surrounding provinces. The hospital has a 400-bed capacity and a 5,200 m^2 garden for use by the patients during remission. Since the majority ($n = 52$) of the patients were in a remission period of their disorder, consent was obtained directly from them. For the remaining patients, a consent was obtained from their relatives ($n = 2$) or legal guardians ($n = 2$). Data concerning age, sex, and number of hospitalizations and follow-up periods were obtained from the patients' medical records and from the person giving consent in each case.

Leprosy patients: Forty-three leprosy patients (28 males, 15 females; age range between 30 and 74 [59.35 ± 11.73]) were included in this study. All were hospitalized at Elazig Leprosy Hospital, which is a regional hospital in Eastern Anatolia serving 18 surrounding provinces. The hospital has a 265-bed capacity and a 38.000 m^2 garden for the patients. Informed consent was obtained directly from the patients. Data concerning age, sex, and number of hospitalizations and follow-up periods were obtained from the patients themselves and their medical records.

Chronic hepatitis B and C patients: This group consisted of 51 chronic hepatitis B (30 males, 21 females; age range between 21 and 62 [38.4 ± 5.3]) and 47 chronic hepatitis C (29 males, 18 females; age range between 17 and 67 [38.4 ± 5.3]) patients who were under follow-up. In the diagnosis of chronic hepatitis B, HBsAg positivity lasting more than 6 months and the absence of IgM antibody to hepatitis B core antigen (anti-HBc) were taken as inclusion criteria (17).

Collection of blood samples: Venous blood samples (5 ml) were taken from each subject in all groups. Serum was separated and stored at −80°C until use.

Serological markers of hepatitis B and C infection: Hepatitis B virus serological markers (HBsAg, anti-HBs, HBeAg, anti-HBe, IgM anti-HBc, anti-HBc total) and anti-HCV (DiaSorin, Vercelly, Italy) were studied by commercial ELISA kits in accordance with kit procedure using Tecan Genesis RMP 100/4 (Zurich, Switzerland) equipment. Anti-HIV was examined by the ELISA method and kits (HIV-1/HIV-2; Abbott Co., North Chicago, Ill., USA).

Determination and genotyping of GBV-C/HGV by reverse transcription nested (RT-n)-PCR assay: GBV-C/HGV genotyping was performed by PCR as previously described by Naito and Abe (18). Briefly, RNA was extracted from 100-μl serum samples by EZ-RNA Total RNA Isolation kit (Biological Industries Co., Beit Haemek, Israel). The 5′ untranslated region (UTR) of the GBV-C/HGV genome was amplified with universal primers (HG1 and HG1R). Two second-round PCRs were then carried out for each sample, using the common universal antisense primer (HG2R) and two different mixtures (Mix A and B) including the type-specific sense primer pairs. A 2-μl aliquot of the first PCR product was subjected to two tubes containing the second sets of each of the inner primer pairs, deoxynucleotides, Taq DNA polymerase and PCR buffer under the same PCR parameters as in the first reaction. In order to distinguish genotypes 2 and 4 by agarose gel electrophoresis, we amplified an amplicon 161 bp in length which is the same size as genotype 4, as a marker, using pSV2-Cat cloning vector (accession number: M777788) as a template with the primer 1, 5′-AATGTTGAGAGTCAGCAGTAGCC-3′, and primer 2, 5′-GAATCAGTA GTTTAACACAT-3′, that bind to pSV2-Cat cloning vector at the 4450 and 4611 positions, respectively. Amplified products were visualized after electrophoresis through a 3% agarose gel stained with ethidium bromide.

Detection of TTV DNA by PCR: n-PCR was performed with two sets of primer pairs. N-22 primer pairs were generated from the N-22 region of the open reading frame (ORF)-1 of TTV DNA (16). Briefly, 40 μl of the TTV DNA pellet was used as a template for first PCR with NGO59 (sense: 5′-ACA GAC AGA GGA GAA GGC AAC ATG-3′) and NGO63 (antisense: 5′- CGT GCA TTT TAC CAT TTC CAA AGT T-3′) primers. Then, 3 μl of the first PCR product was used as a template for semi-nested PCR with NGO61 (sense: 5′-GGC AAC ATG TTA TGG ATA GAC TGG-3′) and NGO63 primer sets. UTR primer pairs were deduced from the 5′ NCR of the viral genome as previously described by Takahashi et al. (19).

Restriction fragment length polymorphism (RFLP) analysis: In order to determine the genotypes of TTV, specific restriction enzymes (*Nde*I, *Pst*I, *NIa*III and *Mse*I; New Engand BioLabs, Beverly, Mass., USA) were used for semi-nested PCR products. Digested products were run through agarose gel and analyzed under UV light (20).

Statistical analysis: Mann-Whitney U test, chi-square and Fisher's exact chi-square test were used with the SPSS 10.01 package program for statistical analysis of the data.

RESULTS

The prevalence and genetic analysis of TTV were determined using the primers' UTR and ORF1 regions of TTV, respectively. The PCR results obtained from UTR and ORF1 are shown in Table 1. TTV DNA was detected in 118 of the 410 sera tested, giving an overall prevalence of 28.7%, and GBV-C/HGV-RNA was detected in 17 of the 410 sera (4.1%). For the genetic analyses of GBV-C/HGV isolates, we analyzed GBV-C/HGV 5′NCR sequences for all samples obtained from the study population. Genetic analyses of TTV isolates was performed using all samples obtained from the study population.

Identification of restriction patterns obtained by restriction enzyme analysis: Digestion of G1 with *Nde*I resulted in 169- and 102-bp fragments, and digestion of G5 resulted in 183- and 88-bp fragments. Digestion of G2 with *Pst*I resulted in 147- and 124-bp fragments. TTV isolates, which were digested by neither *Nde*I nor *Pst*I, belonged to G4 or G6 and were distinguished using restriction digestion by *Nla*III, which yielded 156- and 115-bp fragments. The results of PCR tests for the GBV-C/HGV and TTV genomes, and genotype distribution are reported in Tables 2 and 3, respectively.

Blood donors: Twenty-one of the 125 (16.8%) donor subjects tested positive for TTV DNA. No positivity for GBV-C/HGV was observed in this group. Ten and five of the TTV samples belonged to G2 and G1, respectively. Mean blood donation in this group was 1.64 ± 0.24.

Mentally retarded children: Twenty-seven of 88 (30.6%) mentally retarded children tested positive for TTV DNA and 3 (3.4%) tested positive for GBV-C/HGV RNA. RFLP analysis of all TTV samples indicated that 6, 11, 2 and 1 samples were classified as G1, G2, G3 and G4, respectively. HGV genotyping by PCR revealed all three samples belonged to G2. The mean illness and institutionalization periods in this group were 14.3 ± 4.5 and 5.3 ± 1.3 years, respectively.

Schizophrenic patients: Fifteen of 56 (26.7%) schizophrenic patients tested positive for TTV DNA and 1 (1.7%) tested positive for GBV-C/HGV RNA. Three and 5 of the TTV samples belonged to G1 and G2, respectively. The sample that tested positive for HGV was classified as G2. Duration of illness was longer than 10 years in all cases, and

Table 1. Prevalence of TTV viremia with primers for ORF1 and UTR in the study population

Study population	TTV viremia	
	Primers with ORF1	Primers with UTR
	No. positive/total No. (%)	No. positive/total No. (%)
Blood donors	15/125 (12.0)	21/125 (16.8)
Mentally retarded children	20/88 (22.7)	27/88 (30.6)
Schizophrenic patients	8/56 (14.2)	15/56 (26.7)
Leprosy patients	10/43 (23.2)	14/43 (32.5)
Chronic hepatitis B patients	10/51 (19.6)	16/51 (31.3)
Chronic hepatitis C patients	15/47 (31.9)	25/47 (53.1)
Total	78/410 (19.02)	118/410 (28.7)

Table 2. TT virus DNA and GB-C/HG virus RNA prevalence and coinfection in study population

	Blood donors		Mentally retarded children		Schizophrenic patients		Leprosy patients		Chronic hepatitis B patients		Chronic hepatitis C patients	
	n	*n*+ (%)	*n*	*n*+ (%)	*n*	*n*+ (%)	*n*	*n*+ (%)	*n*	*n*+ (%)	*n*	*n*+ (%)
HGV/GBV-C RNA	125	–	88	3 (3.4)	56	1 (1.7)	43	1 (2.3)	51	4 (7.8)	47	8 (17.0)
TTV DNA	125	21 (16.8)	88	27 (30.6)	56	15 (26.7)	43	14 (32.5)	51	16 (31.3)	47	25 (53.1)
HGV/GBV-C RNA + TTV DNA	125	–	88	–	56	–	43	–	51	–	47	–

n: Number of samples, *n*+: Number of positive samples.
–: A minus sign denotes a negative test.

Table 3. TT virus and GB-C/HG virus genotypes in study population

Study population	*n*	HGV/GBV-C genotypes			*n*	TTV genotypes				
		G1	G2	Other		G1	G2	G3	G4	Other
Blood donors	–	–	–	–	15	5	10	–	–	–
Mental retarded children	3	–	3	–	20	6	11	2	1	–
Schizophrenic patients	1	–	1	–	8	3	5	–	–	–
Leprosy patients	1	–	1	–	10	3	5	2	–	–
Chronic hepatitis B patients	4	–	4	–	10	3	5	2	–	–
Chronic hepatitis C patients	8	–	8	–	15	5	6	3	1	–
Total	17	–	17	–	78	25	42	9	2	–

n: Number of samples.
–: A minus sign denotes a negative test.

the mean institutionalization period in this group was 6.14 ± 1.1 years.

Leprosy patients: Fourteen of 43 (32.5%) leprosy patients tested positive for TTV DNA and 1 (2.3%) tested positive for GBV-C/HGV RNA. Genetic analysis of the TTV samples showed that 3, 5 and 2 samples were classified as G1, G2 and G3, respectively. Genotyping results of the positive GBV-C/HGV sample also belonged to G2. Of leprosy patients, 38 were lepromatous, 3 had tuberculoid leprosy and 2 had borderline leprosy. Duration of illness was more than 10 years in all cases, and the mean institutionalization period was 19.6 ± 5.2 years.

Chronic hepatitis B and C patients: Four of the 51 patients (7.8%) with chronic hepatitis B and 8 of the 47 patients (17.0%) with chronic hepatitis C tested positive for GBV-C/HGV RNA. All samples (4 samples from hepatitis B patients and 8 from hepatitis C) were identified as G2. Sixteen of the 51 patients (31.3%) with chronic hepatitis B and 25 of the 47 patients (53.1%) with chronic hepatitis C tested positive for TTV DNA. Genetic analysis of all TTV samples (10 samples from hepatitis B patients and 15 from hepatitis C) revealed that 8, 11, 5 and 1 samples were classified as G1, G2, G3 and G4, respectively.

The highest prevalence of TTV (53.1%) was found among chronic hepatitis C patients, followed by leprosy patients (32.5%), chronic hepatitis B (31.3%), mentally retarded children (30.6%) and schizophrenic patients (26.7%); the lowest prevalence rate of 16.8% was seen among blood donors. We observed no coinfection with GBV-C/HGV and TTV in any groups. When all groups were compared, GBV-C/HGV positivity was found to be significantly higher in chronic hepatitis C patients than in any other groups, and significantly higher in chronic hepatitis B patients than in blood donors ($P < 0.05$). TTV positivity in chronic hepatitis C patients was significantly higher than in blood donors ($P < 0.05$). There were no significant differences in the numbers of positive tests for GBV-C/HGV and TTV according to the study population, age or sex in any of the groups. Similarly, no significant differences were observed in the numbers of positive or negative tests for GBV-C/HGV and TTV according to duration of illness or mean duration of institutionalization in leprosy patients, schizophrenic patients or mentally retarded children.

DISCUSSION

Detection of genomic sequences by PCR has been the primary method of choice used for the laboratory diagnosis of GBV-C/HGV (21). It is generally recognized that the 5′UTR is the most conserved segment of the virus genome and therefore, it is not unusual to find higher positivity rates when that particular region is used for amplification. The prevalence of GBV-C/HGV viremia in volunteer blood donors in our study was lower (0%, none) than the reported prevalence of 1.5 and 2.0% in volunteer blood donors in the United States and Europe, respectively (4,22,23). To the best of our knowledge, very little work has been carried out concerning the prevalence of GBV-C/HGV in Turkey; thus, it is difficult to state definitively that volunteer blood donors from the Eastern Anatolia region of Turkey are free of or exhibit a low prevalence of GBV-C/HGV infection. The prevalence of GBV-C/HGV among the chronic hepatitis B and C patients in the present study does not appear to be significantly different from previously reported rates ranging from 6.6 to 25.0% in several Asian countries (24,25). The detection rates for hepatitis C patients were significantly higher than those seen in our other study populations. These results indicate that chronic hepatitis B and C patients are also at high risk for GBV-C/HGV infections, possibly via common routes of HBV/HCV transmission, such as blood transfusion (23). In this study, the overall prevalence of exposure to this virus is likely to be greater since only active HGV infection was investigated.

TTV has been found to be extremely common in humans with a prevalence which may exceed 90%, but its detection relies mainly on PCR to amplify viral DNA (19). The wide range of TTV prevalences reported in the world may reflect real differences in prevalence rates or differences in the sensitivity of the PCR method utilized to detect the virus (19,26,27). In the present study, nucleic acids were extracted from a convenience sample consisting of 410 serum samples collected from Turkish residents. The use of N22-PCR protocol gave estimations for the prevalence of TTV infection as 19.0%. With regard to the real prevalence of TTV infection, it is known that the interpretation of the results based on a primer from ORF1 alone can be misleading (28). Indeed, when the samples were re-examined by PCR using UTR-specific primers, a significant number of previously negative samples were identified as false negatives, allowing us to correct the total estimated TTV infection prevalence to 28.7%. This value is lower than those previously reported for Turkish (51.6%), Japanese (92.0%) and Polish (78%) blood donors (19,29,30). In the present study, we found that TTV prevalence was comparable in all tested groups (with the exception of significantly higher values in chronic hepatitis C patients than in blood donors): mentally retarded children (30.6%), schizophrenic patients (26.7%), leprosy patients (32.5%), chronic hepatitis B (31.3%) and C (53.1%) and blood donors (16.8%). Our data thus suggest that infection by TTV in blood donors may not be very frequent in the Eastern Anatolia region. On the other hand, we found no association between TTV infection and institutionalization period or duration of illness. Therefore, it may be concluded that institutionalization, and mental or physical disabilities do not constitute an additional risk for TTV infection.

In the present study, a new, rapid and sensitive genotyping method based on type-specific PCR primers was used in order to determine the GBV-C/HGV genotypes present in this geographical area (18). In view of the present findings, all isolates sequenced belong to genotype 2, the major genotype described in American and European isolates (31). To the best of our knowledge, there is only one report on HGV genotype distribution indicating that genotype 2 is the dominant type among renal transplant recipients in Izmir Province, located in the western part of Turkey (32). Therefore, we believe that this is the predominant genotype in Turkey. The presence of only genotype 2 in Turkey is likely to reflect the European and African origin of the population. Nevertheless, additional investigations using serum samples from other geographic regions of Turkey are required for further classification and characterization of GBV-C/HGV.

Characterization of the TTV genotypes circulating in our study population was carried out by RFLP analysis of N22 nucleotide sequences (20). The analysis of all N22 nucleotide sequences provided evidence for the existence of four major genotypes, G1, G2, G3 and G4, with most belonging to G2 and G1. Nonetheless, the detected diversity of viral genotypes may well be underrepresented because our

analysis was based on RFLP analysis of DNA sequences amplified using N22-specific primers, which may fail to amplify TTV strains other than those from genotypes 1 - 6 (16,20,26,33). Three major genotypes (1, 2 and 3) that are prevalent worldwide were also recognized in this study. The prevalent TTV genotype was found to be G2, which is consistent with results reported for Western Anatolia, where it appears to be more common (29). The present report is the first in the literature to report the G3 and G4 genotypes in Turkey. G3 is detected primarily in Europe (34) while G4 is predominantly found in Asia (20). It is conceivable that the virus was introduced to the eastern part of Turkey through its close geographical proximity to Asian countries, while the western parts of the country are distinct. In addition, a considerable population from the region where this study was performed works in European countries. Further studies will elucidate the role of this diverse and highly prevalent virus in Turkey.

In conclusion, our results indicate a low endemicity of GBV-C/HGV and TTV infection in the Eastern Anatolia region of Turkey. This study also demonstrates that the prevailing GBV-C/HGV genotype in the studied region is G2. The presence of strains belonging to G2 reveals the limited genetic diversity of the GBV-C/HGV circulating in Turkey. We suggest that TTV infection by genotypes 1 and 4 is prevalent in the same region. We also conclude that institutionalization and mental or physical disabilities do not constitute an additional risk for TTV infection.

REFERENCES

1. Tucker, T. J. and Smuts, H. E. (2000): GBV-C/HGV genotypes: proposed nomenclature for genotypes 1-5. J. Med. Virol., 62, 82-83.
2. Tucker, T. J., Smuts, H. E., Eickhaus, P., Robson, S. C. and Kirsch, R. E. (1999): Molecular characterization of the 5′ non-coding region of South African GBV-C/HGV isolates: major deletion and evidence for a fourth genotype. J. Med. Virol., 59, 52-59.
3. Katayama, K., Kageyama, T., Hoshino, F. B., Fukushi, S., Kurihara, C., Ishiyama, N., Okamura, H. and Oya, A. (1998): Full-lengh GBVC/HGV genomes from nine Japanese isolates: characterization by comparative analyses. Arch. Virol., 143, 1063-1075.
4. Roth, W. K., Waschk, D., Marx, S., Tschauder, S., Zeuzem, S., Bialleck, H., Weber, H. and Seifried, E. (1997): Prevalence of hepatitis G virus and its strain variant, the GB agent, in blood donations and their transmission to recipients. Transfusion, 37, 651-655.
5. Wejstal, R., Manson, A. S., Widell, A. and Norkrans, G. (1999): Perinatal transmission of hepatitis G virus (GB virus type C) and hepatitis C virus infections-a comparison. Clin. Infect. Dis., 28, 816-821.
6. Stark, K., Doering, C. D., Bienzele, U., Pauli, G., Hamouda, O., Engel, A. M. and Schreier, E. (1999): Risk and clearance of GB virus C/hepatitis G virus infection in homosexual men: a longitudinal study. J. Med. Virol., 59, 303-306.
7. Komatsu, H., Fujisawa, T., Inui, A., Sogo, T., Morinishi, Y., Miyagawa, Y. and Inui, M. (1999): GBV-C/HGV infection in children with chronic hepatitis C. J. Med. Virol., 59, 154-159.
8. Lampe, E., de Oliveira, J. M., Pereira, J. L., Saback, F. L., Yoshida, C. F. and Niel, C. (1998): Hepatitis G virus (GBV-C) infection among Brazilian patients with chronic liver disease and blood donors. Clin. Diagn. Virol., 9, 1-7.
9. Khudyakov, Y. E., Cong, M. E., Bonafonte, M. T., Abdulmalek, S., Nichols, B. L., Lambert, S., Alter, M. J. and Fields, H. A. (1997): Sequence variation within a nonstructural region of the hepatitis G virus genome. J. Virol., 71, 6875-6880.
10. Mushahwar, I. K., Erker, J. C., Muerhoff, A. S., Leary, T. P., Simons, J. N., Birkenmeyer, L. G., Chalmers, M. L., Pilot-Matias, T. J. and Desai, S. M. (1999): Molecular and biophysical characterization of TT virus: evidence for a new virus family infecting humans. Proc. Natl. Acad. Sci. U.S.A., 96, 3117-3187.
11. Okamoto, H. and Mayumi, M. (2001): TT virus: virological and genomic characteristics and disease association. J. Gastroenterol., 36, 519-529.
12. Peng, Y. H., Nishizawa, T., Takahashi, M., Ishikawa, T., Yoshikawa, A. and Okamoto, H. (2002): Analysis of the entire genomes of thirteen TT virus variants classifiable into the fourth and fifth genetic groups, isolated from viremic infants. Arch. Virol., 147, 21-41.
13. Abe, K., Inami, T., Asano, K., Miyoshi, C., Masaki, N., Hayashi, S., Ishikawa, K., Takebe, Y., Win, K. M., El-Zayadi, A. R., Han, K. H. and Zhang, D. Y. (1999): TT virus infection is widespread in the general populations from different geographic regions. J. Clin. Microbiol., 37, 2703-2705.
14. Prescott, L. E. and Simmonds, P. (1998): International collaborators global distribution of transfusion-transmitted virus. New Engl. J. Med., 339, 776-777.
15. Nishizawa, T., Okamoto, H., Konishi, K., Yoshizawa, H., Miyakawa, Y. and Mayumi, M. (1997): A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. Biochem. Biophys. Res. Commun., 241, 92-97.
16. Okamoto, H., Nishizawa, T., Kato, N., Ukita, M., Ikeda, H., Iizuka, H., Miyakawa, Y. and Mayumi, M. (1998): Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. Hepatol. Res., 10, 1-16.
17. Han, S. H. and Martin, P. (2001): Acute viral hepatitis. p. 148-153. *In* Schlossberg, D. (ed.), Current Therapy of Infectious Disease. Mosby, Inc., Philadelphia.
18. Naito, H. and Abe, K. (2001): Genotyping system of GBV-C/HGV type 1 to type 4 by the polymerase chain reaction using type-specific primers and geographical distribution of viral genotypes. J. Virol. Methods, 91, 3-9.
19. Takahashi, K., Hoshino, H., Ohta, Y., Yoshida, N. and Mishiro, S. (1998): Very high prevalence of TT virus (TTV) infection in general population of Japan revealed by a new set of PCR primers. Hepatol. Res., 12, 233-239.
20. Tanaka, Y., Mizokami, M., Orito, E., Ohno, T., Nakano, T., Kato, T., Kato, H., Mukaide, M., Park, Y. M., Kim, B. S. and Ueda, R. (1998): New genotypes of TT virus (TTV) and a genotyping assay based on restriction fragment length polymorphism. FEBS Lett., 437, 201-206.
21. Tacke, M., Kiyosawa, K., Stark, K., Schlueter, V., Ofenloch-Haehnle, B., Hess, G. and Engel, A. M. (1997): Detection of antibodies to a putative hepatitis G virus envelope protein. Lancet, 349, 318-320.
22. Alter, H. J. (1996): The cloning and clinical implications of HGV and HGBV-C. New Engl. J. Med., 334, 1536-

1537.
23. Linnen, J., Wages, J., Zhang-Keck, Z. Y., Fry, K. E., Kraeczynski, K. Z., Alter, H., Konin, E., Gallagher, M., Alter, M., Hadziyannis, S., Karayiannis, P., Fung, K., Nakatsuji, Y., Shih, J. W. K., Young, L., Piatak, M., Jr., Hoover, C., Fernandez, J., Chen, S., Zou, J. C., Morris, T., Hyams, K. C., Ismay, S., Lifson, J. D., Hess, G., Foung, S. K. H., Thomas, H., Bradley, D., Margolis, H. and Kim, J. P. (1996): Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. Science, 271, 505-508.
24. Panda, S. K., Panigrahi, A. K., Dasarathy, S. and Acharya, S. K. (1996): Hepatitis G virus in India. Lancet, 348, 1319.
25. Sugai, Y., Nakayama, H., Fukuda, M., Sawada, N., Tanaka, T., Tsuda, F., Okamoto, H., Miyakawa, Y. and Mayumi, M. (1997): Infection with GB virus C in patients with chronic liver disease. J. Med. Virol., 51, 175-181.
26. Okamoto, H., Takahashi, M., Nishizawa, T., Ukita, M., Fukuda, M., Tsuda, F., Miyakawa, Y. and Mayumi, M. (1999): Marked genomic heterogeneity and frequent mixed infection of TT virus demonstrated by PCR with primers from coding and noncoding regions. Virology, 259, 428-436.
27. Debiaggi, M., Zara, F., Sacchi, P., Bruno, R., Mazzucco, M., Poma, R., Raffaldi, F., Gerace, L., Perini, M., Pistorio, A., Romero, E. and Filice, G. (2000): Transfusion-transmitted virus infection in HIV-1-seropositive patients. Clin. Microbiol. Infect., 6, 246-250.
28. Mizokami, M., Albrecht, J., Kato, T., Orito, E., Lai, V. C., Goodman, Z., Hong, Z. and Lau, J. Y. N. (2000): TT virus infection in patients with chronic hepatitis C virus infection effect of primers, prevalence, and clinical significance. J. Hepatol., 32, 339-343.
29. Erensoy, S., Sayiner, A. A., Türkoglu, S., Canatan, D., Akarca, U. S., Sertöz, R., Özacar, T., Batur, Y., Badur, S. and Bilgiç, A. (2002): TT virus infection and genotype distribution in blood donors and a group of patients from Turkey. Infection, 30, 299-302.
30. Grabarczyk, P. and Brojer, E. (2002): Polymorphism of the TT virus and its frequency in Polish blood donors. Vox Sang., 82, 177-181.
31. Hino, S. (2002): TTV, a new human virus with single stranded circular DNA genome. Rev. Med. Virol., 12, 151-158.
32. Simmonds, P., Davidson, F., Lycett, C., Prescott, L. E., Mac Donald, D. M., Ellender, J., Yap, P. L., Ludlam, C. A., Haydon, G. H., Gillon, J. and Jarvis, L. M. (1998): Detection of a novel DNA virus (TTV) in blood donors and blood products. Lancet, 352, 191-195.
33. Okamoto, H., Akahane, Y., Ukita, M., Fukuda, M., Tsuda, F., Miyakawa, Y. and Mayumi, M. (1998): Fecal excretion of a nonenveloped DNA virus (TTV) associated with posttransfusion non-A-G hepatitis. J. Med. Virol., 56, 128-132.
34. Erensoy, S., Zeytinoglu, A., Goksel, S., Ozacar, T., Ozkahya, M., Ok, E., Tuumlrkoglu, S. and Bilgic, A. (2002): GB virus C/hepatitis G virus infection among renal transplant recipients in Izmir, Turkey: Molecular analysis of phylogenetic groups. Int. J. Infect. Dis., 6, 242-243.