

GB virus C Viremia and Anti-E2 Antibody Response Among Hemodialysis Patients in Gorgan, Iran

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Background: GB Virus C is a blood-borne virus and a member of *Flaviviridae*, like hepatitis C that is distributed globally and puts hemodialysis patients at high risk of developing liver disease. The clinical significance of GBV-C in this population remains unclear.

Objectives: The current study aimed to evaluate GBV-C infection among hemodialysis patients.

Patients and Methods: Totally, 149 patients receiving hemodialysis were included in the study. The detection of GBV-C sequences in plasma was done by the nested Reverse transcription polymerase chain reaction (RT-PCR) using specific primers selected from highly conserved regions of 5' UTR of GBV-C and antibodies to the envelope protein of GBV-C (anti-E2 GBV-C antibody) were analyzed by also serological methods. In addition, Hepatitis B surface antigen (HBsAg), Hepatitis B core antibody (HBcAb) IgM, anti-Hepatitis C virus (HCV) and anti-hepatitis E virus (HEV) Ab was determined in patients who were GBV-C RNA and anti-E2 GBV-C antibody positive.

Results: The total prevalence of GBV-C infection was 14.7% (95%CI: 0.09-0.21) among patients receiving hemodialysis. The rate of GBV-C viremia and anti-E2 antibody positivity were 6.04% and 10.73%, respectively. Among the subjects who were positive for GBV-C, 27.27% (95% CI: 0.02-0.09), 45.45% (95% CI: 0.03-0.11), 59.9% (95% CI: 0.06-0.16) and 0% (95% CI: 0.01-0.07) were positive for anti-HCV, anti-HBsAg, anti-HBc IgM and anti-(HEV) Ab, respectively. In addition, the rate of both anti-HBc IgM /anti-HCV/ HBsAg and anti-HBc IgM /anti-HCV positivity in GBV-C infected cases were 9.09%. The liver enzymes were normal in all of them. There was significant difference between GBV-C exposures with viral hepatitis co-infection, but there was no correlation between GBV-C exposure with gender, age, ethnicity, time on dialysis and history of blood transfusions. A relatively high frequency of positivity GBV-C-exposure among hemodialysis patients suggested that the transmission route for GBV-C may be nosocomial transmission, and via transfusions.

Conclusions: The current study found a relatively high frequency of positivity GBV-C-exposure among the patients receiving hemodialysis in the area under study. Nosocomial transmission seems to be the main route of GBV-C infection in the area.

Keywords: Hemodialysis; GBV-C viremia; Anti-E2 GBV-C; Iran

1. Background

The GB virus C is a newly identified human RNA virus which belongs to the *Flaviviridae* family (1). The GBV-C genome encodes a polyprotein of approximately 3' 000 amino acids that contains at least two structural proteins as Envelope protein 1 (E1) and Envelope protein 2 (E2) and also non-structural proteins NS2 to NS5 (2). It is closely related to the hepatitis C Virus (HCV) but is more common than HCV among healthy blood donors (3). Transmission via the blood-borne route is the commonest mode, although vertical and sexual transmission is well documented (4, 5). GBV-C is distributed globally; its prevalence in the general population is tenfold higher in African countries than in non-African countries (4). Patients on maintenance hemodialysis are at increased risk of the novel GBV-C infection (6). In these patients, GBV-C viremia is persistent for many years with uncommon recovery (7).

Hepatocarcinogenicity of GBV-C is an important key question that remains controversial (1, 4, 8). The reports seem conflicting. A few investigations demonstrated their association with hepatitis and cirrhosis of the liver and possible presence in hepatocellular carcinoma. It was also observed in hematological disorders and malignancies (9). Several reports have noted an association between GBV-C and hepatitis-associated aplastic anemia besides other hepatitis causing viruses (10). Nevertheless, Most of the results showed that GBV-C infections are asymptomatic, transient, and self-limiting, with slight or no elevation of alanine aminotransferase (ALT). These findings suggested that GBV-C itself is not an important cause of hepatitis, and co-infection with GBV-C does not alter the clinical course of community acquired hepatitis A, B or C (4). Meanwhile, interestingly, GBV-C co-infection has a beneficial effect on survival and progression to Human

Immunodeficiency Virus Infection (AIDS) in patients infected with Human Immunodeficiency Virus (HIV) (9, 11). Nevertheless, an association between GBV-C and HBV and pathogenesis of NHL (non-Hodgkin's lymphoma) could propose the potential participation of this viral infection in the pathogenesis of NHL (12). Therefore, further investigations are necessary to provide more information.

2. Objectives

The current study aimed to assess the relationship between the prevalence of GBV-C RNA and that of antibody to the putative E2 protein (anti-E2) among the patients receiving hemodialysis referred to the Fifth Azar Medical Centre in Gorgan, Iran, and also to decide reappraisal to screen blood supplies for transfusion-transmitted infection agents such as GBV-C.

3. Patients and Methods

3.1. Patient Samples

This cross sectional study was carried out in a hemodialysis unit in Gorgan, Iran, from June 2012 to July 2012. The study and sampling was approved by the Ethics Approval Committee of Faculty of Medical Sciences, Golestan University of Medical Sciences under No.1005-900428-03, on July 2012 and the informed consent was obtained from the patients. Plasma from all 149 (census method) patients receiving hemodialysis were aliquoted and stored at -20°C and -70°C until use. Demographic and other medical information were obtained from their medical records.

3.2. RNA Extraction

Briefly, RNA was extracted from 200 µL of EDTA (Merck, Germany) anticoagulated plasma by a commercially available kit (High Pure Extraction Kit; Roche Diagnostics GmbH, Mannheim, Germany). Negative (GBV-C RNA negative plasma) and positive (GBV-C RNA positive plasma) controls were included in each run.

3.3. GBV-C Reverse transcription polymerase chain reaction (RT-PCR)

The nucleic acid was used directly for reverse transcription polymerase chain reaction (RT-PCR) for cDNA synthesis by a commercial Transcriptor First Strand cDNA Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). The cDNA was synthesized from 1.75 µL of extracted RNA at 25°C for 10 minutes, at 50°C for one hour, and at 85°C for five minutes. Each 10 µL RT master mixture contained 1mM dNTP, 60 µM random hexamer primer, 10 U transcriptor reverse transcriptase, 20U RNase inhibitor, 2.5 µM anchored oligo (dT) primer and 2 µL 5X RT buffer.

3.4. Nested-PCR Reaction

polymerase chain reaction (PCR) amplification was per-

formed for the presence of GB virus C RNA with primers HG1, HG1R for the outer primer pairs (262 bases) and HG2, HG2R for the inner primer pairs (188 bases) from highly conserved regions of 5'untranslated region (5' UTR) of GBV-C PNF2161 isolate based on the reference sequences from GeneBank, as described elsewhere (13, 14). The sequences and positions of the primers used are shown in Table 1. All PCR contamination precautions were observed; and negative controls using sera from subjects with no GBV-C markers were obtained from Digestive Disease Research Institute of Shariati Hospital, Tehran University of Medical Sciences, Iran. Polymerase chain reaction procedure was carefully optimized.

The first-round PCR amplification was performed in a 25 µL reaction volume, containing 1 µL cDNA, 10 pmol of each of the universal outer forward and reverse primers (Table 1), 0.2 mM of each deoxynucleotide (dNTP) (Genet Bio (A type), Korea), 1.25 U of Taq DNA polymerase (Genet Bio (A type), Korea), 1.5 mM MgCl₂ (Genet Bio (A type), Korea) and 2.5 µL 10X PCR buffer (Genet Bio (A type), Korea). The reactions were performed in a thermal cycler (PeQlab, Erlangen, Germany). The reagents for the second PCR round were the same as the ones used in the first round but the used template amount was 0.5 µL. For the second PCR round, the PCR master mixture contained: Reactions were performed in a Peq Lab thermal cycler (Primus Advanced 96 thermal cycler, USA) programmed as follows: 95°C for five minutes; 30 cycles of 94°C for 50 seconds, 55°C for 40 seconds, and 72°C for 50 seconds; and 72°C for five minutes. Amplification was also done for the second round; 95°C for five minutes; 25 cycles of 94°C for 50 seconds, 70°C for 40 seconds, and 72°C for 50 seconds with a final extension at 72°C for five minutes.

3.5. Detection

The PCR products were electrophoresed in a 2% agarose gel, stained with ethidium bromide, and visualized by UV illuminator (Figure 1).

3.6. Serological Markers

Anti-E2 antibody of sera was measured by an enzyme linked immunosorbent assay (GBV-C Ab ELISA kit, Rapidtest, USA). Of these, the positive GBV-C RNA + and anti-E2 GBV-C/GBV-C + sera were tested for hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb IgM), hepatitis C antibody (anti-HCV), and hepatitis E antibody (anti-HEV) by commercial enzyme-linked immunosorbent assay (DS- EIA- HBs-0.01; Fourth Generation Elisa Kit-Italy, DS- EIA-ANTI-HCV; Fourth Generation Elisa Kit- Italy, HBcAb IgM-DiaPlus Inc; Third Generation Elisa Kit- Italy and HEV Ab-DiaPro. Inc., and Third Generation Elisa Kit- Italy respectively). Results were interpreted precisely, according to the manufacturer's instructions.

3.7. Statistical Analyses

Prevalence and 95% confidence intervals (CI) were cal-