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HEPATITIS C VIRUS AS CAUSE OF FULMINANT HEPATITIS–SEQUENCE ANALYSIS OF THE 5' NONTRANSLATED REGION.

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Hepatitis C virus (HCV) contains a highly variable RNA genome with up to 35% differences in the nucleotide sequence. Phylogenetic analyses of nucleotide sequences of the genome of HCV revealed six different types and even more subtypes of this virus. These analyses also showed that variability of the HCV genome is not uniform. To be specific, the nontranslating ends [5'non-translated region (NTR) and 3'NTR], which have a regulatory role in transcription and replication of the virus, are conservative (Van Leeuwen, 2004; Simmonds, 2004). Contrary to that, so-called hypervariable regions (HVR1 and HVR2), which encode the main antigenic determinants, occur in the envelope protein gene of this virus (Smith et al., 1995; Simmonds, 2004). Due to the existence of HVR, a persistent immune response is obstructed in HCV infection, which leads to a chronic condition that can develop into cirrhosis and hepatocellular carcinoma. However, early stages of infection are most often with a sub-clinical picture, because HCV is believed to be noncytopathic and hepatitis is mediated by host immune responses (Fagan and Harrison, 2000; Simmonds, 2004).

In rare cases, HCV can be detected in persons with fulminant hepatitis (FH), the most severe form of acute hepatitis, characterized by massive hepatocyte necrosis, development of hepatic encephalopathy, and high mortality. In cases of FH with HCV infection, the most frequent additional conditions are: hepatitis B virus co-infection, immune suppression, hematological disease, conditions requiring or chemotherapy (Fagan and Harrison, 2000). Some results obtained for FH with HCV infection (FHC) without additional risk factors indicate that the characteristics of viral isolates can influence the pathogenesis of this disease. Farci et al. (1999) inoculated virus isolated from a patient with FHC into a chimpanzee, which induced an unexpectedly severe form of FHC. In addition, Kato et al. (2001) compared the complete nucleotide sequence of the HCV isolate from a patient with FHC with six viral isolates from patients with chronic hepatitis C of the same 2a genotype. The greatest diversity was found in 5'NTR, although this is the most conservative region in the HCV genome. The authors concluded that the influence of the nucleotide structure of 5'NTR on virus replication and translation of virus proteins could be crucial in the development of fulminant hepatitis (Kato et al., 2001).

In this study, we analyzed the genome of HCV isolate from a patient with FHC and compared the 5'NTR with other isolates of the same genotype from patients with chronic hepatitis C in Serbia.

A 59-year-old female patient underwent resection and reconstruction of an abdominal aneurism with graft interposition in 2001. The procedure and the postoperative period had no complications. Before this, liver function and serologic marker tests (for HCV, HBV and HIV) were normal. Alcohol abuse, hepatotoxic drugs, and hypoxia were also excluded. During the second postoperative week, the patient suffered from loss of appetite, nausea, vomiting and fatigue. Marked elevation of alanine aminotranferase (ALT = 2919 U/L, normal <40 U/L), hyperbilirubinemia (total bilirubin = 366 μmol/L, normal <17 μmol/L), and coagulopathy (prothrombin time = 60 s, normal 15 s) were noticed during the first laboratory check, on the 10^{th} day of illness. During the second week, the icterus progressed, and hepatic encephalopathy with marked somnolence and severely disturbed verbal responses developed. On day 17 of the illness, the amount of HCV RNA was 10^5 copies/mL, as detected by a commercial PCR test (Cobas Amplicor HCV Monitor tests, Roche Diagnostic Systems, Mannheim, Germany). The appearance of anti-HCV antibody (seroconversion) happened after day 20. The PCR test then showed a drop of viremy by one level of magnitude and 1 week after that viremy had became undetectable (Fig. 1).

The patient recovered completely within 3 months. The most important survival predictor in FH without liver transplantation is the hepatic encephalopathy grade (65% with encephalopathy I and II survive and 20% with encephalopathy III and IV). Other predictive factors for poor prognosis are age (younger than 10 years and older than 40 years), the level of total bilirubin (>300 μmol/L), hyperbilirubinemia lasting more than 7 days before the occurrence of hepatic encephalopathy, and a prolonged prothrombin time (>50 s) (Sherlock and Dooley, 2002). Our patient developed grade II hepatic encephalopathy and the prior duration of hyperbilirubinemia was less than 7 days, which were favorable prognostic factors. However, age, bilirubin concentration and coagulopathy were poor prognostic factors.

We compared the 5'NTR nucleotide sequence of HCV isolates from this FHC patient with the 5'NTR nucleotide sequences of ten other isolates obtained from patients with chronic HCV infection in Serbia (Blast research program). Briefly, the

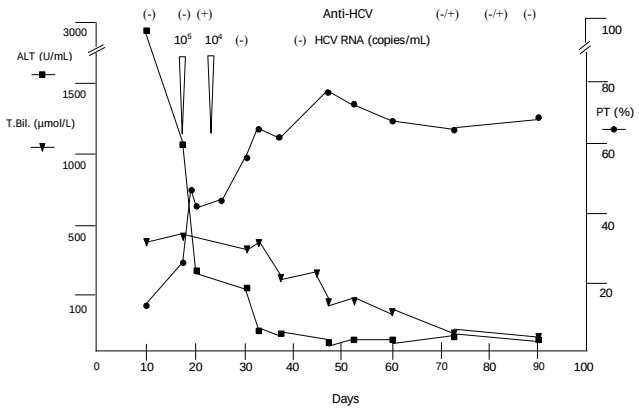

Fig. 1. Biochemical, serological, and molecular profiles of fulminant hepatitis C in the infected patient. The results were obtained 10 days after the first symptoms (ALT - alanine aminotranferase, T.Bil.- total bilirubin, PT - prothrombin time).

HCV genome was isolated with TRIzol™-reagent (Gibco-BRL, Life Technologies). The 5'NTR amplicon, 319 bp in length, was obtained with a One Step RT-PCR Kit (QIAGEN GmbH, Germany) according to the manufacturer's instructions and a universal HCV primer (V i l l a m i l et al., 1995). Automated gene sequencing of the 5'NTR of the HCV genome was done using Big Dye ver.3.1 (MWG DNA Biotech Company, Germany).

The 5'NTR sequence of the isolate from our patient was the same as the prototype of 1b genotype HCV J4 (D10750). Identical nucleotide sequences were found in eight cases obtained from patients with chronic hepatitis C, while two isolates showed a C→T (204 nt) nucleotide substitution. Within this same subtype, the nucleotide sequence of 5'NTR was different in 1 to 5% of nucleotides. Besides the change (C→T) at 204 nt detected here, nucleotide substitutions G→A at 243 nt, C→A at 204 nt, C→T at 120 nt, etc. have been recorded in different isolates of subtype 1b from subjects affected by chronic hepatitis C (B u k h et al., 1992; S m i t h et al., 1995; S t a m e n k o v i ć et al., 2001). It can therefore be said that the examined isolate has an almost identical nucleotide sequence of 5'NTR-a as isolates of the same subtype from 10 chronic carriers of infection.

In HCV certain differences in the nature and development of disease have been detected between different genotypes. However, on the basis of the unclear currently available results it is difficult to decide if these differences should be included in the pathogenesis of fulminant hepatitis. Patients infected with different viral genotypes (1a, 1b, 2a) have developed FHC (F a r c i et al., 1996, 1999; K a t o et al., 2001). These differences could be due to worldwide differences in the distribution of genotypes. The HCV isolate from our patient was the 1b genotype, which is the most common genotype in our country with 49% frequency (S t a m e n k o v i ć et al., 2000).

During the period of the severe form of illness, when hepatic encephalopathy grade II developed, the viral load was 10^5 copies/mL (Fig. 1). However, in chronic forms of HCV infection the viral load is most often from 10^5 to 10^7copies/mL, which means that the number of HCV copies in the blood plasma of our patient was within the range established for chronic carriers of HCV infection (F a g a n and H a r r i s o n, 2000).

Besides characteristics of the HCV isolate, the route of infection and/or physiological state of postoperative trauma can also influence the development of FHC (W o o d f i e l d, 1991; S a c h e r and M e l p o l d e r, 1991; F a r c i et al., 1996). Most probably, our patient was infected with HCV via blood transfusions received during the surgery because the blood pools were tested for anti-HCV antibody, but the presence of HCV RNA was not tested by PCR which can detect HCV RNA in infected blood donors many weeks before appearance of the antibody (H i t z l e r and R u n k e l, 2001). However, the way in which the pattern of infection can influence the development and progress of the disease is not completely clear, even though it has been established that severe forms of it develop most frequently after virus transfer in transfusions (W o o d f i e l d, 1991; F a g a n and H a r r i s o n, 2000).

It can be concluded that HCV can be the sole cause of fulminant hepatitis. The 5'NTR in the HCV isolate obtained from our patient had the same nucleotide sequence as the majority of isolates of this virus from the plasma of patients with chronic hepatitis. The importance of particular isolates, HCV genotypes, and/or viral load can be determined only after studying more patients with FHC and after comparative analyses of the complete nucleotide sequence, which is the aim of our further studies.

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