



How to Diagnosis the Occult Hepatitis C Virus?

Okült (Gizli) Hepatit C Virüs Tanısı Nasıl Olmalı?

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Dear Editor;

Occult hepatitis C (OHC) virus infection, defined as the presence of hepatitis C virus (HCV) RNA in the liver and in peripheral blood mononuclear cells (PBMCs) in the absence of detectable viral RNA in serum, can be found in anti-HCV positive patients with normal serum levels of liver enzymes and in anti-HCV negative patients with persistently elevated liver enzymes of unknown etiology. OHC has been described using highly sensitive nucleic acid amplification assays with a sensitivity of <3 IU/mL. Different studies have revealed that HCV RNA can persist and replicate in immune cells but the relevance of its presence and persistence over time is still unknown. As the contribution of this extrahepatic reservoir could have several clinical implications in viral transmission, treatment response and disease pathogenesis, future studies are required to improve our knowledge of the extrahepatic manifestations of HCV infection and its possible consequences (1).

Individuals with OHC usually have normal serum liver enzymes and most of them are reactive for anti-HCV antibodies. These individuals could have a history of resolved chronic hepatitis C due to antiviral therapy, spontaneous recovery from hepatitis C or asymptomatic exposure to HCV. Low levels of HCV RNA have also been detected in a significant portion of patients with persistently elevated liver enzymes of unknown etiology that were anti-HCV antibody nonreactive (2). The data gathered using highly sensitive assays showed serum HCV-RNA positivity in the majority of persons with a sustained virologic response (SVR) or after spontaneous recovery from hepatitis C in those who were

repeatedly negative for HCV RNA by standard clinical assays (3). Some studies using a highly sensitive real-time polymerase chain reaction (RT PCR) assay have shown the presence of residual HCV RNA in a small number of individuals up to 5 years after apparent spontaneous or treatment induced viral clearance. In addition to sera, HCV RNA was detected in PBMCs. In a large study among 400 patients with SVR, 98% had undetectable hepatic HCV RNA, while only 2% (7 patients) had detectable hepatic HCV RNA. In this study, it could be noticed that the detection of OHC virus was related to the presence of high pretreatment viral load. This factor is well known as prognostic indicator of viral response to antiviral therapy (4).

The principle of HCV RT PCR in PBMC: For PCR performed in PBMCs, five mL of blood was aspirated in sterile tubes containing EDTA, mixed well and lymphocytes were separated by centrifugation on a density gradient (i.e., Ficoll-Hypaque, Pharmacia Biotech) (3). The separation of PBMCs consists of a series of steps. Initially, 3 mL peripheral blood sample is collected into an EDTA-containing tube from each individual. Ficoll hypaque solution is added into a gel-free tube. Peripheral blood samples are transferred to this tube slowly and centrifuged at 1600 rpm for 15 minutes. In the middle of the tube, a cloudy appearance occurred is PBMCs, 1 mL of these cells is transferred to falcon tube by micropipette. Four mL phosphate buffered saline is added and centrifuged at 1300 rpm for 10 minutes. This washing process is repeated four times to purify the cells. Inactivated fetal calf serum is added into the 50 mL of RPMI 1640 medium, 1 mL of this broth is transferred to Eppendorf tubes, 100 µL of 10% dimethyl sulfoxide (DMSO)

is added and this mixture is transferred into the cells as a last step. PBMCs are kept at -80°C until use. Another peripheral blood sample collected into EDTA-containing tube from each individual is centrifuged then plasma is stored at -80°C and used for PCR. RNA isolation procedure is applied to the plasma samples and PBMCs, HCV RNA is extracted from the plasma samples and PBMCs by using RT-PCR. Measurement range of the PCR test must be 25-3.91x10⁸ IU/mL and the sensitivity of the test is 25 IU/mL (5).

The principle of HCV real-time PCR: It is a highly sensitive assay that combines simultaneous amplification and fluorescence detection of target nucleic acid. The fluorescence signal generated during PCR is directly proportional to the target amount in the sample. A synthetic internal control is stabilized within the nucleic acid extraction tubes to be co-purified with the HCV target nucleic acid. HCV genotypes are amplified with similar efficiency applying probes and primers specific for a subsequence of the HCV 5' untranslated region. Primers used are designed to detect the positive sense RNA. Amplification of HCV RNA in samples and internal control RNA is measured independently at different wavelengths due to probe labeling with different fluorescence reporter dyes (HCV RNA; FAM, Internal control RNA; Yakima Yellow). The detection limit of this assay was found to be 3 synthetic HCV RNAs per PCR run (1).

HCV RNA in PBMCs is recommended to detect residual infection in patients with SVR, especially in those with high serum HCV RNA levels before treatment (6). Future works should deal with the possible incidence of OHC or B infections and their pathologic and infective relevance specially in drug abusers, healthcare workers, patients who had received multiple blood transfusions, and patients on hemodialysis, etc. to know the prevalence and spread of this infection in these populations (1,7).

Ethics

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Authorship Contributions

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