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HCV RNA in peripheral blood mononuclear cells (PBMCs) as a predictor of the response to antiviral therapy in chronic hepatitis C

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KEYWORDS

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Abstract *Background:* Hepatitis C virus (HCV) has been found to infect peripheral blood mononuclear cells (PBMCs), using them as a reservoir, which might contribute to the development of resistance to treatment.
Objectives: To study hepatitis virus C (HCV) RNA in peripheral blood mononuclear cells (PBMCs) of patients with chronic HCV infection, and explore the relationship between the HCV RNA in the PBMCs and response to interferon (IFN) therapy.
Methods: Twenty-five patients with chronic viral hepatitis C were included. The HCV RNA in PBMCs and serum was detected after 12 weeks of initializing interferon treatment, at the end of treatment, and 24 week and 1 year follow up after the end of the treatment. At the end of the treatment course, patients who were found to have positive PCR test for HCV RNA in PBMCs were subdivided into two groups, one group continues to receive IFN therapy while the other group stops. The HCV RNA in PBMCs and serum was detected by RT-PCR using the Amplicor HCV 2.0 assay.
Results: All patients had negative serum PCR test for HCV RNA at the end of treatment, nevertheless HCV RNA was detected in PBMCs of approximately 32% of these patients. Patients who tested positively for HCV RNA in PBMCs at the end of treatment had an overall significantly

Abbreviations: HCV, hepatitis C virus; RNA, ribonucleic acid; PBMCs, peripheral blood mononuclear cells; IFN, interferon; RT-PCR, reverse transcriptase-polymerase chain reaction; SVR, sustained viral response.

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higher relapse rate (50%) when compared with patients who tested negatively for HCV RNA in both serum and PBMCs at the end of treatment (6%). Patients with positive HCV RNA in their PBMCs who continue to receive interferon based treatment for further six months had a lower relapse rate (25%) when compared with similar patients who stopped interferon treatment at the 48th week (75%).

Conclusion: Detection of HCV RNA in PBMCs may be important to assess the virological response to interferon treatment and to predict relapse after antiviral therapy and may be taken as a reference to formulate the duration of antiviral therapy in chronic hepatitis C.

1. Introduction

The resolution of hepatitis C, evidenced by normalization of liver function and disappearance of hepatitis C virus RNA from serum as determined by conventional laboratory assays, reflects virus eradication. But in interferon treated patients the HCV RNA in serum sometimes could not show the virus in cells.[1]

Although hepatitis C is mainly hepatotropic, some studies suggest that hepatitis C virus (HCV) infects peripheral blood mononuclear cells (PBMCs), using them as a reservoir, which might contribute to the development of resistance to treatment.[2]

Several factors have been found to influence and predict the response of chronic HCV patients to interferon therapy, such as virus genotype,[3,4] HCV RNA contents in serum,[5] HCV specific cellular immunities after treatment,[6] state of liver disease, baseline body weight, age, sex, and race.[7–9]

Overall, the data accumulated in recent years highlight not only the need for development and implementation of more sensitive HCV RNA diagnostic assays but also the importance of screening both serum and peripheral immune cells for HCV RNA.[10]

The aim of the work was to study hepatitis virus C (HCV) RNA in peripheral blood mononuclear cells (PBMCs) of patients with HCV infection, and explore the relationship between the HCV RNA in the PBMCs and response to interferon (IFN) therapy.

2. Patients and methods

Twenty-five patients with chronic viral hepatitis C were selected from outpatient clinic and inpatient ward of tropical medicine department, Main University Hospital, Alexandria University during 1/7/2010–1/9/2010. The patients were 17 males and 8 females and range of age was from 38 to 47 years old (Table 1). All patients gave informed consent to participate in the study, and the ethics committee approved the protocol. All patients were subjected to thorough history taking and clinical examination, routine laboratory investigations including CBC, urine, stool and liver function tests.

The diagnosis and inclusion were based on (1) the positivity of anti-HCV in serum for more than six months before starting interferon based treatment. (2) The HCV-RNA in serum was positive before starting interferon treatment. (3) All patients showed initial response to interferon treatment at the 12th week. Exclusion criteria included evidence for hepatitis B virus infection, the presence of any systemic illness other than chronic hepatitis C, the history of previous use of antiviral medicine or immunomodulator, the presence of known factors that might interfere with the response to interferon and lastly patients who had breakthrough or were found to be non responders at the end of treatment.

All patients were proved to be eligible candidates for interferon based therapy for chronic hepatitis C according to the criteria adopted by the National project for treatment of HCV sponsored by the ministry of Health and all were given treatment of pegylated interferon alpha 2a (peg IFNα2a) and ribavirin for 48 weeks without serious side effects. Peg IFN was given subcutaneously at a dose of 180 μg/week together with ribavirin 1000–1200 mg daily, 1000 mg for those who weigh $\leqslant$75 kg and 1200 mg for those who weigh $>$75 kg.

At the end of the treatment course, patients who were found to have positive PCR test for HCV RNA in PBMCs were subdivided into two groups, one group included patients who were willing and capable to extend interferon based treatment for further six months while the other group included patients who were unwilling or incapable to receive interferon treatment after the 48th week.

The HCV RNA in PBMCs and serum was detected after 12 weeks of initializing interferon treatment, after 6 and 9 months of treatment, at the end of treatment, and 24 week and 1 year follow up after the end of the treatment. The HCV RNA in PBMC and serum was detected by RT-PCR using the Amplicor HCV 2.0 assay (Roche Diagnostics). Detection of HCV RNA in PBMCs depended on Trizol extraction of RNA. Trizol reagent is ready-to-use reagent for the isolation of total RNA from cells and tissues. The reagent, a mono-phasic solution of phenol and guanidine isothiocyanate, is an improvement to the single-step RNA isolation method. During sample homogenization or lysis, Trizol reagent maintains the integrity of the RNA while disrupting cells and dissolving cell components. Addition of chloroform followed by centrifugation, separates the solution into aqueous phase and an organic phase. RNA remains exclusively in the aqueous phase. After transfer of the aqueous phase, the RNA is recovered by precipitation with isopropyl alcohol.

3. Results

All patients enrolled in this study showed early viral response to interferon therapy at week 12. Nineteen of them showed complete early viral response (Undetectable HCV RNA in serum), while six showed partial early viral response (HCV