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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

Table 1 Patients' characteristics.

Age	Range: 38–47 y	Mean: 42 ± 2.8 y
Sex	Males: 17	Females: 8
BMI	Mean: 25.2 ± 2.2	
AST	Mean: 48.24 ± 27.45 IU/L	
ALT	Mean: 74.88 ± 50.28 IU/L	
Viral load (PCR quantitative)	Mean: 590,470 ± 748,642 IU/ml	
Liver biopsy	F2: 22 patients	F3: 3 patients

Table 2 The results of HCV-RNA in serum and PBMC after treatment with peg interferon based therapy at weeks 12 and 48.

Group	HCV-RNA in PBMC at 12th week		HCV-RNA in serum at 12th week		HCV-RNA in PBMC at 48th week		HCV-RNA in serum at 48th week	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
$n = 25$	9 (36%)	16 (64%)	19 (76%)	6 (24%)	17 (68%)	8 (32%)	25 (100%)	0 (0%)

Table 3 Comparison between SVR rate in patients positive and negative for HCV RNA at the end of treatment.

	SVR rate at 96th week
Patients negative for HCV RNA at both serum and PBMCs at the end of treatment ($n = 17$)	16/17 (94%)
Patients negative for HCV RNA at the serum but positive in PBMCs at the end of treatment ($n = 8$)	4/8 (50%)
Chi-square test (Fisher's exact test)	$P = 0.0224$ significant

RNA in serum remained detectable but decrease by >2 logs). Only nine patients were found to have negative PCR test for HCV RNA in PBMCs after 12 weeks of treatment (Table 2). The genotype of the virus in all of the patients was genotype 4.

After completion of the treatment (after 48 weeks), all patients showed normalization of ALT and negative testing for HCV RNA in serum, while eight of them (32%) showed positive PCR test for HCV RNA in PBMCs.

Patients with chronic hepatitis C who had detectable HCV RNA in their PBMCs were divided into two groups (four patients each). One group was treated with interferon based therapy for further six months; the other group was only provided conservative treatment because they were unwilling to continue interferon treatment owing to financial reasons or for occurrence of intolerable side effects mainly depression.

HCV RNA in PBMCs of three out of the four patients who continue to receive IFN based therapy becomes undetectable after six months by the end of week 72 (Table 4) and PCR testing for HCV RNA in both serum and PBMC after further six months by the end of week 96 proved also to be negative in these three patients (Table 5). On the other hand, three patients from those who received conservative treatment still had positive HCV RNA in their PBMCs, while only one patient had undetectable HCV RNA in PBMC after six months. Moreover, these three patients relapsed after six months of discontinuation of treatment with positive PCR testing for HCV RNA in both serum and PBMCs.

Most of the patients (94%) who tested negatively for HCV RNA in both serum and PBMCs at the end of treatment at 48th week were found to have sustained viral response (SVR) when PCR testing for HCV RNA in the serum proved negative after one year of treatment cessation.

Patients with positive HCV RNA in their PBMCs at the end of the treatment course had a significantly lower SVR rate

Table 4 The results of negative rate of HCV-RNA in serum and PBMC after 72 weeks of treatment for patients with positive HCV RNA in PBMCs at the end of treatment.

	HCV-RNA in PBMC at 72nd week		HCV-RNA in serum at 72nd week	
	Negative	Positive	Negative	Positive
Patients who continue to receive Interferon based therapy ($n = 4$)	3 (75%)	1 (25%)	4 (100%)	0 (0%)
Patients who received conservative treatment ($n = 4$)	1 (25%)	3 (75%)	1 (25%)	3 (75%)
Total	4	4	5	3
Chi-square test (Fisher's exact test)	$P = 0.24$ not significant		$P = 0.07$ not significant	

Table 5 The results of negative rate of HCV-RNA in serum and PBMC at 96th week for patients with positive HCV RNA in PBMCs at the end of treatment.

	HCV-RNA in PBMC at 96th week		HCV-RNA in serum at 96th week	
	Negative	Positive	Negative	Positive
Patients who continue to receive Interferon based therapy ($n = 4$)	3 (75%)	1 (25%)	3 (75%)	1 (25%)
Patients who received conservative treatment ($n = 4$)	1 (25%)	3 (75%)	1 (25%)	3 (75%)
Total	4	4	4	4
Chi-square test (Fisher's exact test)	$P = 0.24$ not significant		$P = 0.24$ not significant	

Table 6 The results of SVR rate and relapse rate in different groups of patients at 72nd and 96th weeks.

	SVR		Relapse rate	
	72nd week	96th week	72nd week	96th week
All patients together ($n = 25$)	22/25 (88%)	21/25 (84%)	3/25 (12%)	4/25 (16%)
Patients negative for HCV RNA at both serum and PBMCs at the end of treatment ($n = 17$)	16/17 (94%)	16/17 (94%)	1/17 (6%)	1/17 (6%)
Patients negative for HCV RNA at the serum but positive in PBMCs at the end of treatment ($n = 8$)	5/8 (63%)	4/8 (50%)	3/8 (37%)	4/8 (50%)
Patients who continue to receive Interferon based therapy ($n = 4$)	4/4 (100%)	3/4 (75%)	0/4 (0%)	1/4 (25%)
Patients who received conservative treatment ($n = 4$)	1/4 (25%)	1/4 (25%)	3/4 (75%)	3/4 (75%)

(50%) when compared with those who had undetectable HCV RNA in their PBMCs (Tables 3 and 6). Consequently, higher relapse rates (50%) of patients who tested positively for HCV RNA in PBMCs were found compared to those with negative HCV RNA in their PBMCs (6%) at the end of treatment.

Moreover, those patients with positive HCV RNA in their PBMCs at the end of the treatment course who continue to receive IFN treatment for further six months had a higher SVR rate (75%) and a lower relapse rate (25%) when compared with those who received conservative treatment (Tables 5 and 6). Although there is a big difference in the SVR rates and relapse rates between patients who continue to receive IFN treatment and those who received conservative treatment, this difference was not found to be statistically significant which may be attributed to the small number of the sample.

4. Discussion

Although hepatocytes are considered to be primary targets of HCV, clinical and experimental evidence strongly indicates that the virus also invades and replicates in cells of other organs, particularly the immune system.[11,12]

In this study, all patients were found to have negative PCR testing for HCV RNA in their sera at the end of the course of interferon based treatment. At the same time, HCV RNA was still detected in freshly isolated peripheral blood mononuclear cells of approximately 32% of these patients. This finding is in accordance with the results reported by Cavalheiro[2] et al. who postulated that the absence of HCV in the serum of patients with chronic hepatitis C by the end of treatment does not mean that there is no circulating virus. HCV in mononuclear cells may be an indicator of the persisting infection. Gallegos-Orozco et al.[13] found that HCV RNA was detected in PBMCs of 20% of individuals with clinical SVR. He postulated that viral persistence and, specifically, the presence of HCV RNA in PBMCs may lead to HCV reactivation under special circumstances, such as immuno suppression.

In agreement to the results of the present study, Zayed et al. reported the presence of detectable HCV RNA in the PBMC of 27% of patients despite the clearance of serum HCV RNA. During follow-up, 80% of the patients who became serum HCV positive 6 months after the end of treatment had a detectable level of HCV RNA in PBMC at the end of treatment. They concluded that the absence of HCV in the serum of patients by the end of treatment does not exclude future viremia. The patient might still be a source of infection to others and they were recommended to testing for HCV in PBMC to detect lack of response to treatment and persisting infection.[14]

Similarly, Gong et al. reported that HCV is capable of infecting and replicating in PBMCs. HCV plus strand RNA as well as minus strand RNA and HCVNS5 protein were found in PBMCs of 62.9%, 40.0% and 85.0% of chronic hepatitis C patients respectively. The patients with minus strand HCV RNA in PBMCs showed a significantly lower 6-month sustained response to IFN, suggesting that minus-strand (a viral replicative form) HCV RNA in PBMCs may be one of the factors influencing response to IFN therapy.[15]

Patients included in the present study showed an overall high SVR rate (84%) which may be attributed to the proper

selection of patients (young age, low viremia, no obesity and early stage of liver disease) and the standard of care provided for them. Moreover, an even higher SVR rate (94%) was noticed in patients who tested negatively for HCV RNA in both serum and PBMCs at the end of treatment at the 48th week. This may give us a clue to the role played by the persistence of HCV RNA in PBMC on the final outcome of the treatment. On the other hand, those patients who tested positively for HCV RNA in PBMCs at the end of treatment had a lower SVR rate (50%) when compared with patients who cleared the HCV RNA from their PBMCs. In other words, patients who tested positively for HCV RNA in PBMCs at the end of treatment had a 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%).

Relapse following end of treatment of antiviral therapy is common. Some authors suggested that patients "cured" after antiviral treatment with persistent viral response may have occult HCV infection in the liver or PBMCs as they demonstrated that HCV can finish replication within PBMCs.[16] However, this concept is not generally accepted as Halfon and coworkers[17] questioned the existence of occult HCV infection as they have not detected HCV RNA in the peripheral blood mononuclear cells (PBMCs) of patients with cryptogenic liver diseases. However, negativity for HCV RNA in PBMCs does not exclude the existence of occult HCV infection because the "gold standard" method to identify this occult infection is by the detection of viral RNA in liver cells. Moreover, when negative data are reported, the sensitivity of the assay is critical to ensure that the lack of HCV RNA detection is not due to the low sensitivity of the technique used.[18,19] Other researchers postulated that contamination or passive adsorption by circulating virus could be the reason for the detection of HCV-RNA in PBMC preparations of chronically infected patients.[20]

Thus HCV in PBMCs may be one of the causes of relapse after antiviral therapy. In certain scenarios including immunosuppression, immunomodulatory therapy or co-infection, instead of eliciting desirable T cell responses in the host, persistent replicating HCV could represent a potential source for virus reactivation, as it has been shown in other viruses, including hepatitis B virus[10] and human herpesvirus 6.[21]

In the present study, follow up for patients showed that patients with positive HCV RNA in their PBMCs who continue to receive interferon based treatment for further six months had a lower relapse rate when compared with patients who stopped interferon treatment at the 48th week. Xu et al.[1] reported that viral relapse was due to nonclearance of virus in cells including hepatocytes and PBMCs although complete response was acquired at the end of treatment. HCV RNA level in serum did not reflect virus situation in cells at the end of IFN treatment of chronic hepatitis C. Moreover, although patients showed complete response at 12 weeks, it did not signify that the patients would acquire sustained viral response.

In 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. Perhaps nonclearance of HCV RNA in PBMCs may be a predictor of unsatisfactory response to antiviral therapy and is a reference to formulate the duration of antiviral therapy in chronic hepatitis C. In this situation prolongation of the interferon treatment may be a recommended strategy, a hypothesis that is in need for further verification.

Conflict of interest

None declared.

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