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Original Article

Study on the Presence of HCV RNA in PBMC Compared with Plasma of Patients with Hepatitis C after Treatment

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Abstract

Background and Aims: Hepatitis C virus is one of the viral infections transmitted by blood transfusion. Patients with thalassaemia frequently need blood transfusion and are in danger of HCV infection. In most cases of infection (85%) the virus evades the immune system and establishes a chronic infection that may lead to cirrhosis and liver carcinoma. Liver is the main site of HCV replication; HCV RNA has been detected in circulating extra hepatic sites, such as in peripheral blood mononuclear cells (PBMC) .It has been proposed that PBMC could be the source of recurrent HCV infection. The aim of this study was to investigate the presence of HCV RNA in PBMCs of thalassaemia patients with hepatitis C after antiviral treatment.

Materials and Methods: About 261 (179 with and 82 without thalassaemia) patients with HCV infection after treatment, 20 patients with HCV infection without treatment and 20 healthy samples as control groups were analyzed in this study. Blood samples were collected in a sterile tube containing EDTA. PBMC were separated from blood of HCV infected patients and control groups by density gradient centrifugation. Viral RNA was extracted from plasma and PBMC by the guanidiumisothiocyonate method. The extracted RNA was amplified by RT-PCR method. Anti-HCV ELISA was performed on all samples.

Results: About 92.7% of HCV infected patients were undetectable for HCV RNA in plasma and PBMC samples after treatment. But HCV RNA was detected in plasma and PBMCs samples 6 of 82 (7.3%) patients with chronic HCV after treatment. After antiviral treatment, 146 of 197 (74.2%) patients with thalassaemia had no detectable HCV RNA and 25.8% (51 of 197) of them had HCV RNA in plasma or PBMCs samples which in two cases, HCV RNA was detected only in PBMC.

Conclusion: More than 90 percent of patients had clearance of HCV RNA in both serum and PBMCs after 5 years of response to antiviral treatment while 74.2% of patients with thalassaemias achieved to SVR after antiviral therapy. Therefore, the presence of the persistent virus in mononuclear cells of patients may cause hepatitis C recurrence at the end of treatment.

Keywords: HCV; sustained virologic response (SVR); RT-PCR; antiviral Treatment

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Introduction

HCV is a single stranded RNA virus of the flaviviridae family that replicates by its negative strand (1-3). It is an enveloped virus with a RNA genome of approximately 9,600 base pairs in length containing an internal ribosomal entry site (IRES) located at the 5'-untranslated region (5'-UTR), which drives the translation of the viral RNA transcript (2, 4, 5). HCV is highly diverged at least six major genotypes and multiple subtypes (6).

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma worldwide (7, 8).

Liver is the main site of HCV replication; HCV RNA has been detected in circulating extra hepatic sites, such as in peripheral blood mononuclear cells (PBMC) .It has been proposed that PBMC could be the source of recurrent HCV infection(9-12).

Combination therapy with pegylated interferon alpha 2 and ribavirin is the current standard of care (SOC) (13, 14).

The goal of treatment of chronic hepatitis C is achieving to sustained virological response (SVR). SVR is assessed 6 months after finishing treatment and is defined as negative HCV RNA in the serum (15-17).

Achievement of an SVR in patients with chronic hepatitis C has been associated with improvements in liver histology and health related quality of life, as well as a reduced risk of hepatocellular carcinoma and liver-related mortality (18).

Thalassaemia is an autosomal recessive disease that due to lack or very low production of hemoglobin could cause destruction of red blood cell. Therefore, blood transfusion is critical for thalassemic patients but it has inevitable side effect e.g. iron overload in heart, liver and endocrine glands.Hepatitis C is one of the health problems in these patients.With blood donors screening by serological and molecular methods, the rate of blood borne HCV infection has reduced. Different studies showed that combination therapy of ribavirin (RIB) with alpha interferon (IFN) is better than mono therapy with IFN in non thalassemic patients. RIB can induce hemolytic anemia in thalassemicpatients. So, the requirement for blood transfusion in these patients will be increased.

The aim of this study was to investigate the presence of HCV RNA in peripheral blood nuclear cells of thalassemic and non thalassemicpatients Suffered from hepatitis C after antiviral treatment.

Methods

Subjects

261 (179 with and 82 without thalassaemia) patients with chronic hepatitis C, referred to the Blood Transfusion Organization Clinical Laboratory, were included in this survey .The patients were 150 males and111 females, mean aged of 32.6 who had history of positive for anti-HCV antibody over six months.

Also, 25 patients with HCV chronic infection those were positive for anti-HCV ELISA test and HCV- RNA RT-PCR in plasma as positive control group were selected. All the patients were treated with pegylated interferon alpha and Ribavirin between 5-8 years. Also, 25 healthy samples as negative control groups were analyzed in this study. All patients signed a written informed consent for the study.

Serologic Tests

All samples were tested for anti-HCV antibody by enzyme-linked immunosorbent assay (ELISA) (Hepanostika HCV ultra, manufactured by Beijing United Biomedical Co. Ltd, China) and confirmed results of anti-HCV positive by recombinantimmunoblot assay (RIBA) (MP Biomedicals, So-lon, OH, United States).

RNA Extraction and cDNA Synthesis

Blood samples were collected in a sterile tubes containing EDTA .Then PBMCs were isolated from blood by centrifugation over a density gradient and stored at -70°C until used.

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Viral RNA was extracted using Trizol reagent (Invitrogen, Karlsruhe, Germany). The quantity and purity of extracted RNA was assessed by measuring absorbance at 260 nm and the ratio A260/A280 in a UV-spectrophotometer (NanoDropInc, Wilmington, DE USA). Reverse transcription of viral RNA into cDNA was performed using random hexamer primer and Takara 1st strand cDNA synthesis kit (Takara, Bio Inc., Shiga, Japan), according to manufacturer's recommendations.

The presence of HCV RNA was detected using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) kit (homemade, ibto, Iran) according to manufacture recommendation.

The data were statistically analyzed using the SPSS software, version 16 (SPSS, Inc., IL, USA) and they were compared using the Chi-square test. Values of $p \leq 0.05$ were considered to be statistically significant.

Results

None of healthy control groups had positive results for HCV RNA in plasma and PBMCs samples. About 92.7% of HCV infected patients after treatment had no detectable HCV RNA. But HCV RNA was detected in plasma and PBMCs samples 6 of 82 (7.3%) patients with chronic HCV after treatment. After antiviral treatment, 146 of 197 (74.2%**)** patients with thalassaemia were undetectable for HCV RNA and 25.8% (51 of 197) of them had HCV RNA in plasmaor PBMCs samples which in two cases, HCV RNA was detected only in PBMC. From 146 subjects with undetectable results, 44% of them had mono therapy with IFN and 46% of them had IFN in combination with RIB. About 43.1% (22 of 51) and 56.9% (29 of 51) of patients with thalassaemia, who were positive for HCV RNA in both plasma and PBMCs samples, had IFN alone or combination therapy with RIB respectively.

Discussion

In our study, 74.2% of patients with thalassaemia and 92.7% of HCV infected patients without thalassaemia had clearance of HCV RNA in both plasma and PBMCs after 5 years of response to antiviral treatment .About 25.8% of HCV infected patients with thalassaemia and 7.3% of patients had HCV-RNA positive results in PBMCs and plasma after treatment. This study suggests that SVR may be considered to show eradication of HCV infection.

Several factors have been found to influence and predict the response of patients with chronic HCV to interferon therapy, such as virus genotype, HCV RNA viral load in serum, HCV specific cellular immunity after treatment, state of liver disease, baseline body weight, age, sex, and race (9, 17, 19).

Treatment of HCV infection in patients with thalassaemia is different. Due to defect in hemoglobin chain, membrane of red blood cells is frigid and susceptible to lysis. On the

Table 1: Distribution of frequency of HCV infected patients according to treatment.

patient groups \ Treatment	HCV-RNA: Undetectable Anti-HCV: Positive	HCV-RNA: Positive Anti-HCV: Positive	Total No	Total %
HCV infected patient	76 (92.7%)	6 (7.3%)	82	(100)
HCV infected patient with thalassemia	146 (74.2%**)**	51 (25.8%)	197	(100)

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other hand, anemia is a major complication for these patients, so they need to blood transfusion repeatedly. This may lead to iron over load which is a negative prognostic factor in antiviral treatment in addition to precipitation in main organ. Ribavirin causes RBC lysis and iron over load. It can worsen anemia in HCV infected patients with thalassemia. So, the main protocol for HCV treatment is interferon-based therapy and in non-responder patients' use of IFN in combination with ribavirin.

Replication of HCV RNA in PBMCs may be the source of relapse after antiviral treatment in patients with chronic hepatitis C (9). Maylin et al investigated none of the 344 patient's included had a relapse during the long-term follow-up evaluation (median, 3.27 y; range, 0.50–18 y). HCV RNA was detectable in 2 of 114 (1.7%) liver tissue specimens and in none of 156 (0.0%) of the PBMC specimens tested. This result strongly suggests that SVR may be considered to show eradication of HCV infection (20).

Also in the study of George et al 150 patients with SVR followed for 5 years, the majority of patients had good outcomes. Serum virological relapse was not seen (21).

Patients included in the Hanno *et al* study showed an overall high SVR rate (84%) 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 (19).

In another study, Sood *et al*. investigated during a followed up of 6 months to 8 years, 8 of 100 patients with initial SVR developed late relapse of HCV infection. Relapse was more common in patients who had cirrhosis (5/28 [18%] vs. (3/72 [4%] without cirrhosis; p=0.037). SVR is durable in most patients, but some patients do have late relapse; long term follow up may be particularly important in a subset of patients with HCV infection who have liver cirrhosis (22).

Persistence of the replicating virus in PBMCs, even if HCV RNA is undetectable in serum, may cause infection reactivation in the future (13).

In the study of Pham et al, both positive and negative strand HCV RNA was detected in PBMC by using mitogen-stimulated lymphocyte cultures for increasing the number of cells containing the persistent virus (23).

In our study, HCV RNA was detected only in two PBMC samples of patients after treatment which indicates the sensitivity of assays for detection of HCV RNA particularly after treatment with low copy of virus must be increased.

Moreover, clearance of HCV RNA in PBMC at the end of IFN treatment was a predictor of durable response to antiviral therapy in patients with chronic hepatitis (24). In a study Pearlman showed that in chronic HCV infection, therapy-induced SVR is a clinically meaningful end point. SVR is a durable marker of viral eradication, because evidence for extra hepatic residual viremia is limited, and multiple reports demonstrate that late relapse is rarely observed; SVR is tantamount to cure (17).

In conclusion, lack of detection of viremia at the end of treatment in serum alone does not indicate absence of the persisting virusin mononuclear cells and patients are likely to be at great risk of hepatitis C recurrence. So, screening on serum and PBMC for HCV RNA is necessary and also the need for development and implementation of more sensitive HCV RNA diagnostic assays is very important.

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