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Value of Quantitative HCV RNA in Management of Chronic Hepatitis C Patients with Genotype 2 and 3

Shahid Sarwar, Arshad Kamal Butt, Altaf Alam, Anwaar A. Khan, Farzana Shafqat, Kashif Malik, Aamir Khan Niazi

Department of Gastroenterology and Hepatology. Shaikh Zayed Postgraduate Medical Institute, Lahore.

SUMMARY

Determination of viral load by quantitative polymerase chain reaction (PCR) for hepatitis C virus (HCV) is part of workup before initiating interferon and ribavirin combination therapy for chronic hepatitis C. This study was carried out to determine predictive value of baseline viral load in patients with viral genotype 2 and 3, for response to therapy. **Material and methods:** Patients with chronic hepatitis C and genotype 2 and 3 were included in study. Viral load was determined before starting treatment with standard interferon and ribavirin for six months in all patients. Patients were checked for end of treatment (EOT) and sustained viral response (SVR) by qualitative PCR for HCV. Response to therapy was correlated with baseline viral load by student's t test. **Results:** Total of 55 patients were included. Male to female ratio was 1.1/1 (29/26). Six patients were of genotype 2, one patient was harboring both genotype 2 and 3 while rest of 48 patients had genotype 3 of hepatitis C virus. Baseline viral load was less than 2 million copies/ml in 25 patients while 30 patients had viral load in excess of 2 millions copies/ml. Treatment was completed in 50 patients. Sustained viral response (SVR) was seen in 31 patients and 19 patients were non-responders. No significant association was found between response to therapy and baseline viral load. **Conclusion:** Pre-treatment viral load is not predictive of response to combination therapy with interferon in patients with genotype 2 and 3.

Key Words: Chronic hepatitis C, Genotype, Quantitative polymerase chain reaction (PCR), Sustained viral response (SVR).

INTRODUCTION

The hepatitis C virus (HCV) is a major public health problem and a leading cause of chronic liver disease.[1] It prevalence varies from 0.01% in United Kingdom to 17-20% in Egypt.[2] Pakistan is an endemic area for hepatitis C with 6.5% population afflicted with hepatitis C.[3]

Spectrum of illness varies from asymptomatic carriers to patients with advanced cirrhosis and catastrophic complications like variceal bleeding, ascites, hepatic encephalopathy and hepatocellular carcinoma.[4,5] Course of this illness has changed remarkably with awareness of its routs of transmission and availability of better treatment modalities. Ever since the advent of combination therapy, cure rate of interferon therapy has improved significantly. Introduction of pegylated interferon in recent past has further enhanced response to therapy.[4]

Before starting therapy, number of investigations are need to access the stage of disease and to determine the chance of favorable response to therapy. These tests include qualitative as well as quantitative HCV RNA by polymerase chain reaction (PCR), baseline liver function tests, genotype of virus, abdominal ultrasound examination and liver biopsy.

Treatment of hepatitis C is expensive, especially for patients in under-developed countries. Costly tests like PCR further add to the cost of therapy. Every effort is being made to cut down the expenses of treatment. Apart from bringing down the cost of drugs, reduction in number of

preliminary tests will further help in this direction.

Baseline viral load of HCV is established using a quantitative HCV RNA assay. Quantitative assays ascertain the quantity of HCV RNA in blood using either target amplification (PCR, TMA) or signal amplification techniques (branched DNA assay). The level of HCV RNA in blood helps in predicting the likelihood of response to treatment, and the change in the level of HCV RNA during treatment can be used to monitor response. [5] Information regarding the viral load may aid in counseling patients as to their likelihood of response.[6]

HCV RNA quantitative is also used for early response prediction at three months of therapy for genotype 1 and 4. Decline of viral load by more than 2 log predicts positive response. So checking for baseline viral load is part of treatment protocol for genotype 1 and 4.

But early response prediction is not needed in genotype 2 and 3 due to good sustained viral response to therapy. Predictive value of baseline viral load for response to therapy in genotype 2 and 3 is also not established. [6] Should we check quantitative HCV RNA in patients with genotype 2 and 3 or is it just adding to the cost of treatment, is the question still awaiting definite answer.

Objective

To evaluate the predictability of quantitative HCV RNA for sustained viral response to combination therapy in chronic hepatitis C.

Study design

It was cohort type of analytical study.

Place of study

Study was carried out at Department of Gastroenterology and Hepatology Shaikh Zayed Post Graduate Medical Institute Lahore from February 2003 to August 2004.

Patient selection

Patients with positive anti HCV by ELISA III were provisionally included in this study. Viremia was confirmed by qualitative PCR (Polymerase Chain Reaction). Method used for Qualitative HCV RNA was Nested PCR based on five major processes, extraction of HCV RNA from serum sample, reverse transcription of target RNA to generate c DNA, two rounds of PCR amplification and detection.

Complete history and physical examination was performed. Baseline investigations including complete blood count, coagulation profile, liver function tests, renal function tests, abdominal ultrasound and upper GI endoscopy, to look for varices where needed were carried out.

Genotyping of virus (Inno-LIPA; Innogenetics NV,Ghent, Belgium) was carried out in all of these patient. Patients with positive PCR and genotype 2 or 3 were included in study.

Patients with positive hepatitis B surface antigen, positive HIV, chronic liver disease due to alcoholic liver disease, hepatotoxic drugs, autoimmune chronic hepatitis and haemochromatosis or patients with signs of hepatic decompensation (variceal bleed, ascites, history of hepatic encephalopathy) were excluded from study group.

MATERIAL AND METHODS

All patients were checked for viral load by quantitative HCV RNA. Quantiplex assay (b DNA) with lower limit of detection 3200 copies/ml was used for determining viral load. Viral load was expressed in copies/ml. HCV RNA qualitative was checked before start of treatment, at end of treatment and six months after stopping treatment for sustained virological response (SVR). SVR was defined as negative qualitative PCR six months after completion of treatment.

Percutaneous liver biopsy was performed prior to therapy in all of these patients. Liver biopsies were paraffin embedded and stained with haematoxylin-eosin. Histological features were scored for inflammation and fibrosis using Knodell scoring system. [7]

All patients were treated with combination therapy, inj interferon 3million units subcutaneous thrice a week along with cap ribavirin 1000-1200mg daily(1000mg for patients < 75Kg body weight and 1200mg for patients with body weight >75Kg) in three divided doses. All patients were advised for contraception during and six months after

completion of treatment. These patients were followed initially fortnightly for one month and then monthly. On each follow up visit, detailed history regarding side effects like fever, fatigue, malaise, hair loss and depression was taken. Complete blood count, coagulation profile, liver and renal function tests and serum uric acid were checked on each visit. Thyroid function tests and pregnancy test in females were checked every three months Post treatment liver functions were checked monthly for six months. Sustained virological response was defined as negative qualitative PCR six months after treatment completion. Treatment plan was in accordance to latest guidelines of The American Association for Study of Liver Diseases (AASLD). [8]

Statistical analysis

Statistical analysis was carried out using a software package (SPSS 10.0.1; SPSS Inc, 1989-1999 Chicago, Ill). Patients with sustained viral response and non-responders were compared for variables like age, sex, body weight, height, body mass index, baseline ALT and histological Knodell score using student's t test. Correlation of baseline viral load and response to interferon therapy was determined using two tail unpaired Student's t test. P value of less than 0.05 was considered significant. Patients were grouped further depending on viral load above or below 2 million copies/ml. Cutoff figure of 2 million copies/ml was chosen based on observations in studies that patients with viral load in excess of two million have poor response to combination therapy. [9] Effect of viral load in excess of or less than 2 million copies on sustained viral response (SVR) was checked using Chi square test.

RESULTS

Total number of patients included in this study was 55. Male to female ratio was 1.11/1 (29/26). Mean age of patients was 34.58 (±10.34). Mean weight of patients was 65.33 Kg (±10.56) while mean height was 163.8cm (±11.10). Mean body mass index was 22.64 (±4.54). Baseline alanine aminotransferase (ALT) was within normal limits in 20 patients, raised but less than double the normal value in 12 and more than double the upper limit of normal in 13 patients. All patients had liver biopsy prior to therapy. Mean knodell score on liver biopsy was 5.9 (±2.16). Only 6 of total 55 patients were of genotype 2 while one patient had both genotype 2 and 3. Remaining 48 patients were harboring HCV of genotype 3. Distribution of subclasses of genotype 2 and 3 is shown in Table 1.

Table 1: Distribution subclasses of genotype 2 and 3.

Genotype	No of patients	Percentage
2a	6	10.9
2a + 3a	1	1.8
3a	33	60
3b	9	16.4
3c	3	5.5
3d	1	1.8
3a + 3b	2	3.6

Mean baseline viral load checked via quantitative PCR was 4.8 million. When patients were grouped depending on viral load more or less than 2 million copies/ml, 25 patients had viral load less than 2 million copies/ml and viral load was in excess of 2 million copies/ml in 30 patients. These two groups of patients were compared for baseline parameters and no significant difference was noted as shown in Table 2.

Interferon therapy was planned for six months in all patients. Fifty patients completed the treatment. Three patients were lost to follow up while treatment has to be abandoned in two patients. Severe bone marrow suppression with pancytopenia on complete blood count and marked depressive psychosis were responsible for stopping treatment in these two patients respectively.

End of treatment response with negative PCR was seen in 35 patients. Sustained viral response was seen in 31 patients while 4 patients had relapse of HCV RNA.

Patients with SVR and those with positive PCR at six months post-treatment were compared for baseline variables other than viral load as shown in Table 3. Both groups were comparable with no significant difference in variables capable of affecting the outcome. Viral load before treatment in patients with and without SVR was not significantly

different. (p 0.478) It can be interpreted that viral load did not have significant impact on response to interferon therapy.

Table 2: **Comparison of patients with baseline viral load more than 2 million copies/ml and patients with viral load less than 2 million copies**

Variable	Patients with Viral load < 2 million	Patients with viral load ≥ 2 million	P value
Mean age (Years)	37.04± 11.52	32.53± 8.92	0.108
Sex			
Males	11	18	0.237
Females	14	12	
Mean Height (cm)	164.45± 10.86	163.38±11.59	0.809
Mean weight (Kg)	66.50±9.86	64.55±11.12	0.551
BMI	22.26±3.6	22.88±5.13	0.744
Baseline ALT (IU/ml)	119.88	118.07	0.946
Mean knodell score	6.08±2.47	5.83 ±1.89	0.672

Table 3: **Comparison of patients with SVR and Non-responders**

Variable	Patients with SVR*	Non-Responders	P value
Mean age (Years)	34.74±10.80	35.26±10.29	0.867
Mean height (cm)	167.35±11.3	156.11±6.41	0.011
Mean weight (Kg)	65.71±7.73	63.46±15.31	0.532
Mean BMI	22.57±3.80	23.34±5.78	0.693
Mean baseline ALT (IU/ml)	127.63	104.26	0.444
Mean viral load (Copies/ ml)	4.7 million	2.6 millions	0.478
Mean knodell score	6.06±2.03	5.79±2.57	0.677

* SVR- sustained viral response (HCV RNA negative at six months post-treatment)

When we compared patients with baseline viral load more than 2 million copies/ml with patients with less than 2 million copies/ml viral load for sustained viral response, response was better in patients with higher viral load but the difference was not statistically significant.(0.186) Table 4.

Table 4: **Correlation of pre-treatment viral load and sustained viral response**

	PCR six months post treatment		Total	Percentage of SVR
	Negative	Positive		
Viral load < 2 million/ml	12	11	23	52.17
Viral load > 2 million /ml	19	8	27	70.37
Total	31	19	50	

DISCUSSION

Viral load has long been used as guide for duration and type of therapy needed.[9] It also helps in prediction of sustained viral response.[10-12] Large pool of studies has favored routine checking for this test before initiating therapy in patients of hepatitis C.

Adinolfi *et al* concluded that degree of liver damage and progression of liver fibrosis is significantly associated with viral load of patient.[13] The lowest levels of hepatitis C viremia are, in general, associated with minimal liver disease. Pretreatment serum HCV RNA level has been found to be associated with chances of sustained viral response.[14-18] Mizokami M et al found baseline viral load to be predictor of viral reponse.[19] Suzuki T et al described b DNA assay useful in clinical management of patients being treated by interferon therapy.[20] Quantitative HCV RNA has been used to recognize patients with early viral response at three months of therapy. [21] Treatment is abandoned in patients with less than desired response. This approach is part of standard practice for patients with viral genotype other than 2 and 3.[2223]

Role of quantitative HCV RNA in patients with genotype 2 and 3 is still far from being established. Bhupinder *et al*, in a study of 50 patients with hepatitis C identified no association between HCV RNA titer and histological injury on liver biopsy.[24] In a study of 58 patients de Araujo concluded that the use of viral RNA quantification as an evolutive predictor or determinant of the

severity of hepatitis C is incorrect and correct interpretation of viral titer should consider a broader context depending on multiple factors that are more complex than the simple value obtained upon quantification. [25] Similar questions regarding value of viral titers have been raised in number of other studies. [26,27]

In the study by Hadziyannis and colleagues, high HCV RNA levels (> 2 million copies/mL) did not adversely affect response rates among patients with genotype 2 or 3 who were treated for a shorter duration and with a lower dose of ribavirin, although it did affect the response rate among those with genotype 1. [28]

According to recommendations given by Commonwealth Minister of Health and Aged Care (MSAC) in March 2000, viral load titer is predictive of a response to interferon therapy, but is not sufficient an indicator that patients should be excluded from a trial of therapy on the basis of the results of viral load testing. These recommendations were prepared after mata-analysis of 35 studies. [29]

Another problem with testing viral load prior to therapy is spontaneous fluctuations in viral copies. In a study by Halfon P et al it was found that Spontaneous fluctuations of HCV RNA ranged from 2.8 to 5.7 fold with branched DNA assay and from 2.9 to 5.6-fold with Monitor. These large spontaneous fluctuations (up to 0.75 log), observed daily, weekly, and monthly, raise doubt about the clinical value of a single assessment of pre-therapeutic viremia.

In our study of 55 patients, we were unable to identify significant correlation between viral load and SVR. We also grouped patients in two groups, one with viral load less than 2 million copies and second group with high b DNA titer. Both groups were comparable with no significant difference in other factors capable of influencing the outcome as is shown in Table 2. Response was paradoxically better in patients with high viral titer but this difference was not statistically significant. Our results are comparable with number of recent studies.

Considering the doubtful association of viral titer with response to therapy and fluctuating nature of viral load, routine checking of quantitative HCV RNA is not needed in patients with genotype 2 & 3.

CONCLUSION

Quantitative detection of viral load is not predictive of response to combination therapy with interferon in patients with genotype 2 and 3.

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The Authors:

Shahid Sarwar
Trainee Registrar
Department of Gastroenterology and Hepatology.
Shaikh Zayed Post Graduate Medical Institute
Lahore, Pakistan

Arshad Kamal Butt
Associate Professor
Department of Gastroenterology and Hepatology.
Shaikh Zayed Post Graduate Medical Institute
Lahore, Pakistan

Altaf Alam
Associate Professor
Department of Gastroenterology and Hepatology.
Shaikh Zayed Post Graduate Medical Institute
Lahore, Pakistan

Anwaar A. Khan
Professor and Head of Department
Department of Gastroenterology and Hepatology.
Shaikh Zayed Post Graduate Medical Institute
Lahore, Pakistan

Farzana Shafqat
Assistant Professor
Department of Gastroenterology and Hepatology.
Shaikh Zayed Post Graduate Medical Institute
Lahore, Pakistan

Kashif Malik
Senior Registrar
Department of Gastroenterology and Hepatology.
Shaikh Zayed Post Graduate Medical Institute
Lahore, Pakistan

Irfan Ahmad
Trainee Registrar
Department of Gastroenterology and Hepatology.
Shaikh Zayed Post Graduate Medical Institute
Lahore, Pakistan

Aamir Khan Niazi
Trainee Registrar
Department of Gastroenterology and Hepatology.
Shaikh Zayed Post Graduate Medical Institute
Lahore, Pakistan

Address for Correspondence:

Shahid Sarwar
153-D Muslim Road Jinnah Colony
Saman Abad, Lahore
nawalshahid@hotmail.com