INTRODUCTION

Hepatitis C Viral (HCV) infection is a global health problem affecting 150 million people worldwide.¹ In Pakistan, an estimated 5% of the population is affected by this disease.² Major risk factors for HCV in Pakistan include excessive use of injections, contaminated needles/medical equipment, unscreened blood transfusions, intravenous drug abuse, dental and surgical procedures like circumcision, ear/nose piercing by quacks and dialysis.³

HCV is diagnosed by detecting anti-HCV antibodies in serum by different methods like Enzyme Immunoassay (EIA), chemiluminescence and immunochromatography. HCV detection and its quantification is the second main step in analysis of HCV which is carried out by amplification techniques⁴ including Polymerase Chain Reaction (PCR). Amplification techniques can detect the actual presence or absence of HCV in blood as well as its quantity and are, therefore, essential before starting the therapy.⁵

HCV viral load is the amount of virus in the blood of the patient. This level is an indication of viral activity in the infected person. It is expressed in International Units per milliliter (IU/ml). Viral load can be Low Viral Load (LVL) or High Viral Load (HVL). Conventionally, a low viral load (LVL) is less than 800,000 IU/ml whereas high viral load (HVL) is more than 800,000 IU/ml.⁶ Viral load is usually determined by PCR, a process involving extraction of Viral Ribonucleic Acid (RNA) from the sample followed by its amplification and detection. Quantitative PCR determines viral load whereas Qualitative PCR indicates the presence or absence of viral RNA.

The standard treatment of HCV includes interferon (IFN) and ribavirin. In Pakistan, standard interferon alpha is being used in a dose of 3 million units subcutaneously thrice weekly and oral ribavirin (10.6 mg/kg body weight) daily for 6 months.⁷ Different outcomes of this treatment include Sustained Virological Response (SVR), no response, partial response, relapse after response and breakthrough phenomenon. These outcomes are detected by PCR results like Early Virological Response (EVR) at 12 weeks of treatment, End-of-Treatment-Response (ETR) at the end of treatment, and Sustained Virological Response (SVR) 6 months after end of treatment.⁸

ETR is the response observed at the end of treatment. There are many host as well as virus associated factors

END-OF-TREATMENT-RESPONSE IN PATIENTS TREATED FOR HEPATITIS C VIRUS WITH STANDARD INTERFERON AND RIBAVIRIN BASED ON VIRAL LOAD

Muhammad Ali Rathore, Agha Babar Hussain and Eijaz Ghani

ABSTRACT

Objective: To determine the End-of-Treatment-Response (ETR) to standard interferon and ribavirin based regimen in patients of chronic hepatitis C and to compare the ETR response in low and high viral load groups.

Study Design: Descriptive study.

Place and Duration of Study: Virology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from March 2012 to May 2013.

Methodology: Patients with chronic hepatitis C virus infection were included in the study. Pre-treatment viral load was determined by RoboGene Quantification kit. Based on viral load, the 400 patients were divided into two equal groups of low viral load (< 800,000 IU/ml) and high viral load (> 800,000 IU/ml). The patients were treated with standard interferon alpha (3 million units subcutaneously thrice weekly) and ribavirin (10.6 mg/kg body weight) for 6 months. ETR was measured using Sacace Biotechnologies Qualitative kit. Chi-square test was used to compare the ETR in the two viral load groups. P-value < 0.05 was considered as significant.

Results: Out of 400 patients, 206 (51.5%) were males and 194 (48.5%) were females. Two hundred seventy (67.5%) patients achieved ETR and 130 (35.5%) failed to do so. In low viral load group, 145 (72.5%) patients achieved and 55 (27.5%) patients did not achieve ETR. In high viral load group, 123 (61.5%) patients achieved and 77 (38.5%) did not achieve ETR. The difference in ETR between low and high viral load groups was statistically significant (p=0.019).

Conclusion: End-of-treatment-response in patients treated for hepatitis C virus with standard interferon and ribavirin was greater in patients with low viral load as compared to patients with high viral load.

which can affect ETR.\textsuperscript{9} One important factor is viral load at the start of treatment which can considerably affect the outcome of treatment.\textsuperscript{10}

A considerable work has been done internationally on viral load but since genotype is different in Pakistan as compared to developed countries, there is a dire need to have our own local data regarding prognostic factors for HCV treatment especially in patients being treated with standard interferon and ribavirin. This study was thus conceived to establish a relationship between viral load and treatment outcome in treatment-naïve patients with HCV infection.

**METHODOLOGY**

The study was conducted in the Department of Virology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from March 2012 to May 2013 after approval of research and ethical committee of the institute. The patients infected with HCV, belonging to both genders, and willing for treatment with standard interferon and ribavirin were included in the study. Patients with coinfection of hepatitis B virus and those treated earlier for HCV were excluded from the study. After a verbal consent, 3 ml of patients sample was collected in Ethylene Diamine Tetra Acetate (EDTA) bottles using aseptic technique. The specimen was centrifuged at 800 - 1600 g for 20 minutes and plasma separated in aliquots for further testing. The plasma samples were stored at -50 degrees Celsius until tested in batches. Frozen samples were not thawed more than once for optimal results. Non-probability consecutive sampling technique was used for sample collection. The sample size was calculated using WHO calculator and was 400 (200 patients in each viral load group).

Pre-treatment viral load was determined by RoboGene HCV Quantification kit. According to the result of viral load, the patients were divided into two equal groups of low viral load (< 800,000 IU/ml) and high viral load (> 800,000 IU/ml). The patients were treated with standard interferon alpha (3 million units subcutaneously thrice weekly) and ribavirin (10.6 mg/kg body weight) for 6 months. End-of-treatment-response was measured using Sacace Biotechnologies HCV Qualitative kit.

The data obtained from the study was analyzed with the help of computer software program SPSS (Statistical Package for Social Sciences) version 17. Descriptive statistics were calculated for both qualitative and quantitative variables. For qualitative variables, including gender and overall end of treatment response in each group, frequencies and percentages were calculated. For quantitative variables like age and viral load in each group, mean standard deviation was calculated. Chi-square test was used to compare the ETR in two viral load groups. P-value < 0.05 was considered as significant.

**RESULTS**

Out of a total of 400 patients, 206 (51.5\%) were males and 194 (48.5\%) were females. In 200 patients with low viral load group, 92 (46\%) were males and 108 (54\%) females. In 200 patients with high viral load group, 114 (57\%) were males and 86 (43\%) females. The age of the patients ranged from 18 to 62 years with a mean of 40.9 ± 10.95 years. The age of patients in low viral load group was in the range of 18 - 62 years with a mean of 39.1 ± 10.8 years. The age of patients in high viral load group was in the range of 18 - 62 years with a mean of 42.8 ± 10.7 years.

Out of 400 patients, 270 (67.5\%) achieved ETR whereas 130 (32.5\%) failed to achieve ETR. In low viral load group, 145 (72.5\%) patients achieved ETR whereas 55 (27.5\%) were unable to achieve ETR (Figure 1). In high viral load group, 123 (61.5\%) patients achieved ETR and 77 (38.5\%) patients did not achieve ETR (Figure 2).

The difference in ETR between low viral load group and high viral load group was statistically significant according to Chi-square test (p = 0.0193, Table I).

![Figure 1: ETR in low viral load group (n = 200).](image1)

![Figure 2: ETR in high viral load group (n = 200).](image2)
In Pakistan, chronic hepatitis C is currently being treated with pegylated as well as standard interferon and ribavirin to prevent complications including cirrhosis and hepatocellular carcinoma. INF along with ribavirin is an essential component of the treatment for patients infected with chronic hepatitis C. In this study, the end of treatment response to standard INF and ribavirin therapy was recorded in relation to viral load in patients. The standard treatment of care in Pakistan for chronic HCV consists of a combined therapy of INF plus ribavirin. The cost of this therapy has a huge impact on the economy of this country, especially the health sector, because a significant portion of Pakistani population (approximately 10 million) is infected with this virus and majority of them are candidates for treatment.

In this study, a high rate of ETR was observed (67.5%) which was similar to other studies done in Pakistan including Aziz et al. (63.4%), Idrees et al. (67%) and Zuberi et al. (70%). This study showed a much higher ETR in comparison to international studies conducted by McHutchison et al. (53%), Hui et al. (57%) and Poynard et al. (57%), which is prevalent in the developed countries and is associated with severe disease and a decrease response to treatment.

A relationship between baseline pre-treatment viral concentration and response to standard INF was also observed in this study. ETR was significantly higher in patients (72.5%) having a low viral load, i.e. with a pre-treatment viral load of less than 800,000 IU/ml as compared to patients (61.5%) with pre-treatment viral load of ≥ 800,000 IU/ml. This relationship was also observed in other similar studies conducted in Pakistan. In a study conducted by Aziz et al., ETR in low viral load group was 68.8% as compared to 60.4% in high viral load group. Similarly, in a study conducted by Idrees et al., ETR was achieved more in patients with low viral load as compared to those with high viral load. This relationship was also observed in a study conducted by Martinot-Peignoux et al. in Europe in which it was demonstrated that the low baseline serum viral load is associated with a significantly higher probability of achieving virological response after INF-based therapy. The results of all these studies suggest that low viral load is favorable for virological response after treatment as compared to high viral load.

In patients with low viral load, further distribution was done according to viral load and it was found that 177 (88.5%) of patients had viral load less than 400,000 IU/ml and 23 (11.5%) of patients had viral load more than 400,000 IU/ml. With the introduction of peg interferon and ribavirin in countries with predominant genotype-I, a new cut off value of 400,000 IU/ml is being proposed which is predictive of rapid virological response and high sustained virological response rates (> 80%) even after shortened duration of treatment according to studies conducted by Mangia et al., Moreno et al., von Wagner et al. and Yu et al. In patients with low viral load, 135 (67.5%) of patients had viral load less than 200,000 IU/ml and 65 (32.5%) had viral load more than 200,000 IU/ml. The ETR in low viral load group was 72.5% which shows that within low viral load group, a better response was seen when majority of patients were below 200,000 IU/ml. This was similar to another study conducted in Pakistan by Idrees et al. in which ETR was significantly higher in patients with pre-treatment viral load of less than 200,000 IU/ml as compared to patients with viral load more than 200,000 IU/ml. Several other recent international studies like Shiffman et al. and Hadyziyannis et al. also indicated the same trend of better ETR in patients with pre-treatment viral load of less than 200,000 IU/ml.

In patients with high viral load, 106 (53%) had viral load more than 2,000,000 IU/ml and 84 (42%) had viral load more than 30,000,000 IU/ml. This shows that in this study, a large number of patients in high viral load group were having viral load towards the higher side of viral load range. This fact is most probably responsible for the low ETR observed in this group (61.5%).

**Table I:** Table comparing ETR in low and high viral load groups.

<table>
<thead>
<tr>
<th>Viral load</th>
<th>End of treatment response (ETR)</th>
<th>Total</th>
<th>p-value (2 tailed significance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ETR achieved (HCV RNA PCR-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low viral load</td>
<td>145 (72.5%)</td>
<td>55</td>
<td>200 (100%)</td>
</tr>
<tr>
<td>High viral load</td>
<td>123 (61.50%)</td>
<td>77</td>
<td>200 (100%)</td>
</tr>
<tr>
<td></td>
<td>Total 268 (67%)</td>
<td>132</td>
<td>400 (100%)</td>
</tr>
</tbody>
</table>

**Table II:** Low viral load group: further distribution of patients according to cut off value of 400,000 IU/ml and 200,000 IU/ml

<table>
<thead>
<tr>
<th>Viral load group</th>
<th>Cut off value 400,000 IU/ml</th>
<th>Number of patients</th>
<th>Percentage of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low viral load</td>
<td></td>
<td>177</td>
<td>88.5%</td>
</tr>
<tr>
<td></td>
<td>Viral load less than 400,000 IU/ml</td>
<td>177</td>
<td>88.5%</td>
</tr>
<tr>
<td></td>
<td>Viral load more than 400,000 IU/ml</td>
<td>23</td>
<td>11.5%</td>
</tr>
<tr>
<td></td>
<td>Total 268</td>
<td>135</td>
<td>67.5%</td>
</tr>
<tr>
<td></td>
<td>Viral load less than 200,000 IU/ml</td>
<td>65</td>
<td>32.5%</td>
</tr>
<tr>
<td></td>
<td>Viral load more than 200,000 IU/ml</td>
<td>65</td>
<td>32.5%</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In Pakistan, chronic hepatitis C is currently being treated with pegylated as well as standard interferon and ribavirin to prevent complications including cirrhosis and hepatocellular carcinoma. INF along with ribavirin is an essential component of the treatment for patients infected with chronic hepatitis C. In this study, the end of treatment response to standard INF and ribavirin therapy was recorded in relation to viral load in patients. The standard treatment of care in Pakistan for chronic HCV consists of a combined therapy of INF plus ribavirin. The cost of this therapy has a huge impact on the economy of this country, especially the health sector, because a significant portion of Pakistani population (approximately 10 million) is infected with this virus and majority of them are candidates for treatment.
Very few studies have been conducted in Pakistan regarding viral load and its effect on ETR and assessment as a prognostic factor. The reason for lack of such studies in Pakistan include the high cost of quantitative PCR and problems regarding longer follow-up of patients taking antiviral therapy. This study shows that apart from other factors affecting response to HCV treatment like age, gender, weight, genotype and race etc., viral load is also an important prognostic factor for virological response. The results of this study can help to modify antiviral therapy individually for infected HCV patients that can reduce economic burden, side effects of antiviral therapy, and better response rates. The results of this study also suggest that HCV quantification should also be performed before initiating antiviral therapy against HCV whenever and wherever possible.

CONCLUSION

It was found that more HCV patients in low viral load group achieved end of treatment response after treatment with standard interferon and ribavirin, as compared to high viral load group.

REFERENCES