# SUSTAINED BIOCHEMICAL AND VIROLOGICAL RESPONSE OF DIFFERENT HCV GENOTYPES TO INTERFERON-ALPHA PLUS RIBAVIRIN COMBINATION THERAPY

Sajjad Iqbal[1], Khalil-Ur-Rehman[1], M Zahoor-Ul-Hassan Dogar[2] Sajid Bashir[3] and Muhammad Shoaib Akhtar[3]

[1]Department of Biochemistry, University of Agriculture Faisalabad, [2]Sargodha Medical College, University of Sargodha, and [3]Department of Pharmacy, University of Sargodha, Sargodha- Pakistan,

**Summary**

Initial and sustained responses of different HCV genotypes to Interferon plus Ribavirin combination therapy were assessed in a Quasi-experimental study. Out of a total of 208 patients, 115(55.29%) males and 93(44.71%) females chronically infected with different HCV genotypes and undergoing Interferon plus Ribavirin combination therapy were followed up to 12-18 months after the end of therapy. Therapy period was 6 months for sensitive genotypes (2a, 2b, 3a and 3b) and 12 months in case of resistant genotypes (1a, 1b and 4) or untypable HCV genotypes. ALT was monitored monthly during and after the therapy. Pre-therapy and mid-therapy quantification was done in resistant and untypable genotypes.

The results obtained showed that out of 208 patients, 155 (74.52%) were responders and 53 (25.48%) non-responders at the end of therapy (EOT). Response was associated with the absence of detectable serum HCV RNA and normal ALT level. Among the responders, sustained viral response (SVR) was noted in 88.35% while the best EOT response was found in the 3b (83.87%) among the sensitive genotypes and in 1b (50%) among the resistant genotypes. It is conceivable; therefore, that combination of Interferon and Ribavirin was efficacious in the patients able to tolerate genotype-specific therapy.

**Key words:** HCV genotypes, Interferon, Ribavirin, combination therapy, Sustained response.

*Address for Correspondence: Prof. Dr. Muhammad Shoaib Akhtar, Department of Pharmacy, University of Sargodha, Sargodha-40100, Pakistan. *Email*: drmsakhtar@gmail.com,

## Introduction

Chronic liver disease is one of the commonest causes of mortality and morbidity world-wide, especially in developing countries (1). It is usually associated with hepatitis C virus (HCV) infection (2). Hepatitis C virus infection causes a slowly progressive disease which can lead to chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma which is a leading cause of death worldwide; particularly in the developing countries (3).

In the past, treatment of HCV infected patients was limited to monotherapy with various types of interferon (i.e., interferon alpha-2a, interferon alpha-2b, etc) that have similar immunomodulatory and antiviral effects. Monotherapy has achieved viral clearance in only 15%–20% (4). The nucleoside analogue ribavirin has also been evaluated in combination with interferon alpha-2b, and this combination of antiviral agents has markedly improved the sustained virological response. Ribavirin as a single agent enhances a type-1 cytokine-mediated immune response but does not suppress viraemia levels; when given in combination with interferon synergistic antiviral effects are seen (5). Spontaneous remission of Hepatitis C disease seems to be rare, but interferon-alpha therapy induces a response characterized by normalization of aminotransferase levels and improvement of liver histological findings in 38% to 48% of patients (6). However, more than half of responders have relapsed and re-increased serum aminotransferase levels within 6 months after withdrawal of interferon-alpha therapy. Less than 20% of treated patients have a sustained response with persistently normal aminotransferase levels during the 6-month period after treatment (7). Long-term outcome in patients with sustained response is not well known. A few studies of small numbers of patients with follow-up periods of 1 to 4 years have suggested a long-term benefit in some patients; however, late relapse was seen in other studies (8). As far as ascertained little information was available on long term biochemical and virological outcome especially in Pakistan, and the question of the long-term benefit of interferon-alpha therapy remained to be answered. To address this question, we have assessed the long-term biochemical and virological outcomes of 208 Interferon plus Ribavirin treated patients with chronic hepatitis C.

## Material and Methods

**Test Patients**: The patients willing to take part in the study were provided all the required information. Those who gave written consent were included in the present study, which was conducted at Shalamar Hospital Lahore, from June 2007 to June 2009. Pretreatment data (address, contact number, family status, age, sex, previous investigations, any other disease and previous surgery) were taken. All the patients were anti-HCV positive by ELISA and confirmed by HCV RNA detection. Pretreatment, ALT level, genotyping including quantification for resistant and untypable genotypes were compulsory, while the ALT levels were monitored monthly in all genotypes. Pre-treatment HBV vaccination was given to all the HCV infected patients but negative for HBsAg (confirmed by ELISA) to reduce the chances of co-infection of HBV.

**Exclusive criteria:** The patients co-infected with HBV or other diseases like TB, diabetes, HIV etc, which can affect the therapy were excluded from the study. The patients who had developed cirrhosis or liver carcinoma were also excluded. To observe

the sustained response, only those patients were included who participated for post therapy follow up, others were excluded from the study.

**HCV RNA Extraction:** HCV RNA was extracted from 200µl of serum, using the protocol of Gentra Kit (PURESCRIPT®, Minneapolis, and MN 55441 USA). The extracted RNA was re-hydrated in 50µl hydrated solution. Reverse transcription (RT) was carried out to convert RNA to complimentary DNA (cDNA) with 10µl of RNA using 1µM of downstream primer and 100U of Moloney–Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) enzyme (Fermentas) in a final volume of 20µl. Amplification of cDNA was done in two PCR rounds using nested PCR. In first round a big fragment of 375 bp from a selected region of HCV genome was amplified. In second round an inner portion of 250 bp from first round fragment was amplified using a nested pair of primers. The PCR products were submitted to electrophoresis using a 1.8% agarose gel in TBE buffer and visualized by ethedium bromide staining under ultraviolet light.

**Genotyping:** For HCV genotyping RNA was extracted according to the kit protocol of Gentra kit (PURESCRIPT®, Minneapolis MN 55441, USA) as mentioned above. 10µl of isolated RNA was converted into complimentary DNA (cDNA). The cDNA was then subjected to two rounds of amplification. The first round of PCR was done with outer primers specific for core region of HCV. The second round of PCR was performed with one universal inner sense and 11 genotype-specific anti-sense primers in a multiplex PCR as described previously (9, 10). The amplified PCR products were electrophoresed on 2% agarose gel stained with ethedium bromide and evaluated on UV light. 50 bp Ladder Marker was also parallel run in the gel to see the proper position of genotype specific fragments with comparison.

## Results

A total of 208 patients (115 males and 93 females) chronically infected with different HCV genotypes were selected in the present study (Fig-1). Age range was 10 to 60 years (Mean=36). Out of 208 HCV patients 10 were infected with resistant HCV genotypes (1a, 1b & 4) and 172 with sensitive genotypes (2a, 2b, 3a and 3b). Twenty cases remained untypable and 06 patients were infected with mixed (more than one) HCV genotypes as demonstrated in Table-1.

74.52% patients out of 208 were EOT responders and 25.48% non-responders. While the sustained virological response remained in 88.35% out of 155 EOT responders, 11.65% relapsed after 12 to 18 months of EOT as revealed in table-1 & 3 and Fig-2. Response was associated with, absence of detectable HCV RNA and normal ALT level. EOT response was high in males 87(75.65%) as compare to females 68(73.12%) as shown in Fig.1. In the same way SVR was also high in males 49(90.74%) as compared to females 42(85.72%) [Fig.2]. For sensitive and resistant genotypes, the EOT response was 79.65% and 40%, while the SVR was 93.26% and 33.34 %, respectively.

In most of the cases related to sensitive or resistant genotypes ALT became normal after half therapy and at the end of therapy in few cases the ALT level was still elevated in sensitive genotypes even the RNA was negative. But the ALT level became normal in all

responding cases after 2 to 3 months of EOT. On the other hand in non- responding cases the situation was contrast. In sensitive genotypes, the high EOT response was seen in 3b, which was (83.87%) followed by 2b (80%), 3a (78.83%) and 2a (70%). While in resistant genotypes 1b was noted highly EOT responding (50%) as compare to 1a (40%) and genotype 4 (33.34%). In untypable and mixed genotypes the EOT response was 55% and 50%, respectively (Table1).

As is evident from Table 2, the EOT response in mixed genotypes involving resistant type was 33.34% and in the mixed genotypes including both sensitive types response was 66.66%. SVR out of four sensitive genotypes (2a, 2b, 3a, 3b) was noted high in genotype 2b (100%), while in remaining three types; 2a, 3a, and 3b the value were 90%, 95.13% and 91.42%, respectively. However, in resistant types, SVR was 50%, 00% and 00% in 1a, 1b and 4, respectively. In untypable and mixed genotypes the SVR response was 66.67% and 50%, respectively. Al these data have been summarized in Table 3.

**Table 1: EOT response of different HCV genotypes**

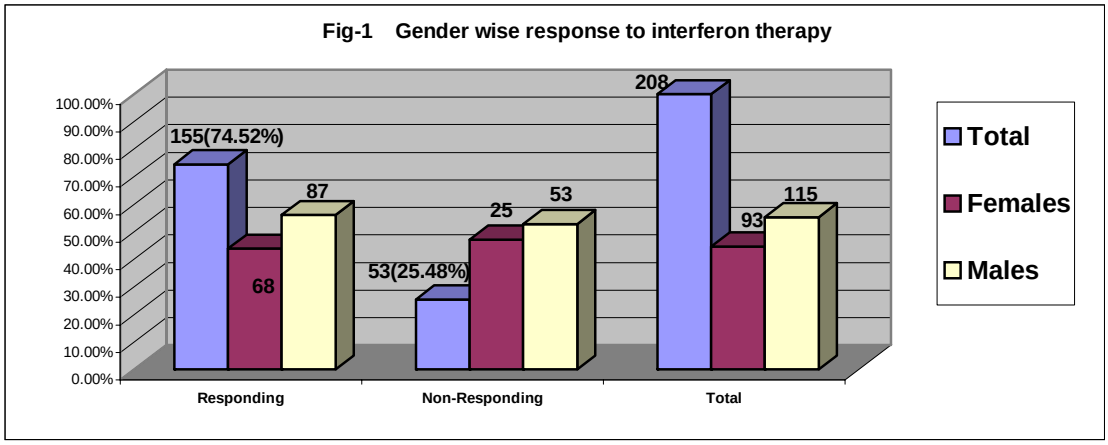
| Genotype | Total Genotypes | Responding Genotypes | Non-Responding Genotypes |
|---|---|---|---|
| **1a** | 05 (2.40%) | 02 (40%) | 03 (60%) |
| **1b** | 02 (0.96%) | 01 (50%) | 01 (50%) |
| **2a** | 20 (9.61%) | 14 (70%) | 06 (30%) |
| **2b** | 05 (2.40%) | 04 (80%) | 01 (20%) |
| **3a** | 85 (40.86%) | 67 (78.83%) | 18 (21.17%) |
| **3b** | 62 (29.81%) | 52 (83.87%) | 10 (16.13%) |
| **4** | 03 (1.44%) | 01 (33.34%) | 02 (66.66%) |
| **Untypable** | 20 (9.61%) | 11 (55%) | 09 (45%) |
| **Mixed** | 06 (2.88%) | 03 (50%) | 03 (50%) |
| **Total** | **208** | **155** (74.52%) | **53** (25.48%) |

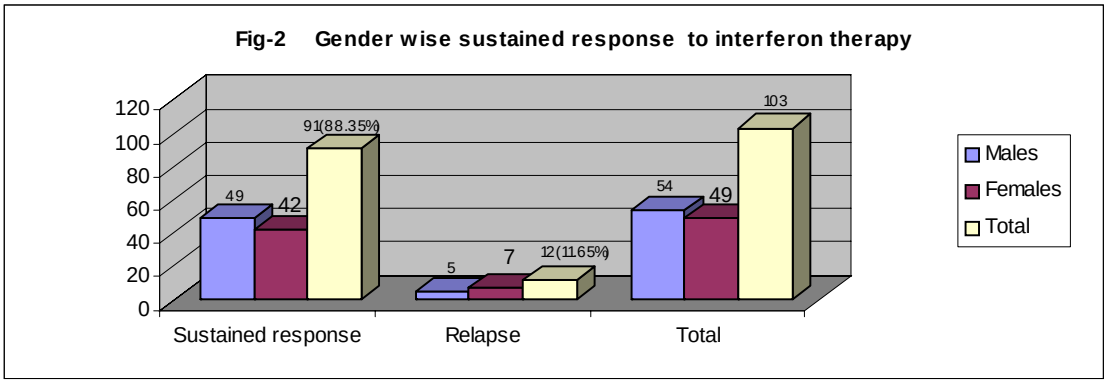
**Table-2: EOT response of mixed genotypes.**

| Mixed Genotypes | Responding | Non-responding | Total |
|---|---|---|---|
| 3a & 2a | 01 | 00 | 01 |
| 3a & 3b | 01 | 01 | 02 |
| 1b & 3a | 00 | 01 | 01 |
| 1a & 3b | 00 | 01 | 01 |
| 1a & 3a | 01 | 00 | 01 |
| **Total** | **03 (50%)** | **03 (50%)** | **06** |

**Table-3: Sustained viral response (SVR) of different HCV genotypes**

| Genotypes | Total | Sustained responders | Relapsed cases |
|---|---|---|---|
| 1a | 02 | 01 | 01 |
| 1b | 01 | 00 | 01 |
| 2a | 10 | 09 | 01 |
| 2b | 03 | 03 | 00 |
| 3a | 41 | 39 | 02 |
| 3b | 35 | 32 | 03 |
| 4 | 00 | 00 | 00 |
| **Untypable** | 09 | 06 | 03 |
| **Mixed Genotypes** | 02 | 01 | 01 |
| **Total** | **103** | **91 (88.35%)** | **12 (11.65%)** |



## Discussion

The present study was carried out to evaluate the impact of interferon plus ribavirin combination therapy on different HCV genotypes. According to our results most of the patients with chronic hepatitis C who have persistently normal serum ALT levels during the 6 months post interferon-alpha therapy and no detectable serum HCV RNA with PCR have long-term biochemical and histological improvement, which has also been mentioned in previous study(11). The absence of detectable HCV RNA in the serum after 6 months of interferon-alpha therapy has suggested possible eradication of HCV infection from blood and liver.

A sustained virological response was seen in 88.35% out of 103 EOT responders, which is low as compare to another study in which the sustained virological response was 96% (11) and high as compared to Kalantari *et al*. (12) and Muhammad *et al*. (13) where the SVR was 75.14% and 77.27%, respectively. According to our findings the patients who were HCV RNA negative and with normal ALT levels during six months of post therapy also remained HCV RNA negative when followed 12-18 months. Thus, the persistence of detectable serum HCV RNA despite normal serum ALT levels probably reflects the persistence of HCV replication with the risk for late relapse, as shown previously (14). The 11.65% rate of late increase in serum ALT levels and HCV RNA detection was seen out of 103 EOT responders in present study which is lower than Muhammad *et al*.(13) and Chemello *et al* (14) findings where the rate was 14.86% to 79%. According to Richard *et al* (8), the relapse rate was 7% which is low as comparing to our findings.

It was also interesting to note that in most of the responding cases ALT level became normal after completion of half therapy and in few cases ALT level remained elevated even at the end of therapy, but all these cases were HCV RNA negative. When these cases were followed after therapy the ALT level became normal. The elevation of ALT in responding cases is still mystery. The same situation has also been recorded in other studies (15, 16). In addition, 69.04% patients with EOT response were 40 years of age or younger, which is in accordance with Paglioro *et al* (17) whose response was 62% in the same age group. With increasing trend of age the EOT response to Interferon plus Ribavirin combination therapy was low in our study as depicted in Table 3, which has also been noted by others (18). It could be perhaps due to weak immune system in aged groups which does not help to wash out the virus from the body.

The high proportion of long-term response was seen in women as compare to men, which suggests that women have a better response than men. The high response in females may be due to complete rest and proper medication as compare to males who have to take part in social and economical activities and could not take proper medication and complete rest. This has been also reported by Michiko *et al* (19). Among EOT responders, genotypes 1a, 1b and 4 were less common (40%, 50% and 33.34%) as compared to genotype 3a, 3b and 2a, 2b in which the EOT response was more common (78.83%, 83.87% and 70%, 80%). This is in accordance with the view that HCV genotypes 1a, 1b and 4 are associated with a poor rate of response to interferon-alpha therapy as compared to genotype 2a, 2b and 3a, 3b (18).

More sustained response was seen in genotypes 2b and 3a (100% and 95.13%) followed by 3b and 2a (91.42% and 90%). Fortunately, in Asia in which our country lies more common genotypes are 2a, 2b and 3a, 3b (20, 21). It must, however, be pointed out that the number of cases of genotype 1a, 1b, 2b and 4 were quite low which demands more study to evaluate the exact results.

Twenty untypable genotypes, included in the present study were treated on trial basis for one year and ALT level was monitored monthly followed by every three month viral load. As demonstrated in table-2 in mixed genotypes including resistant type the EOT response was low as compare to those in which the both genotypes were sensitive. So the mixed genotypes including resistant type should be treated for twelve months and those in which both types are sensitive should be treated till six months. The absence of detectable HCV RNA 12 to 18 months after treatment is consistent with the view that HCV infection may be cleared with interferon-alpha therapy in patients with hepatitis C. It is in confirmation to results of previous studies, in which 86% to 91% of persons with sustained response had no detectable liver HCV RNA 1 to 2 years after therapy (8, 22). The disappearance of serum HCV RNA seems to be correlated with the disappearance of liver HCV RNA. The presence of a low level of HCV RNA in the liver, not detected on PCR, cannot be ruled out as yet. In our study, however, relapses have not occurred among 91 patients without liver HCV RNA during follow-up; suggesting that these patients might have been cured of the disease.

In conclusion, Interferon plus ribavirin combination therapy for HCV patients has proved more effective and persistence of normal serum ALT levels and the absence of detectable serum HCV RNA till 6 months after therapy seemed to be reliable indicators of long-term biochemical and virological remission. However, before therapy HCV genotyping must be taken under consideration to decide the therapy duration. The patients infected with untypable genotypes should be treated on trial bases for 12 months. In mixed genotypes, if there are both sensitive types the therapy duration should be six months, if resistant type is present with sensitive type the therapy duration must be extended to 12 months. Moreover, all the patients must be monitored at least six months after the end of therapy to see the sustained virological response.

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