Hematologic adverse effects and efficacy monitoring in chronic Hepatitis C patients treated with interferon and ribavirin combination therapy

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Abstract: 180 million people are affected by chronic Hepatitis C Virus infection globally and more than 50 million in South East Asia. Combination of Interferon and Ribavirin is the current anti-HCV therapy in practice and is associated with certain hematologic adverse effects. In this concurrent observational study the incidence rate of major hematologic adverse effects and efficacy outcomes of Interferon and Ribavirin combination therapy was evaluated in 288 chronic hepatitis C patients at Lahore General Hospital. Levels of Hb, TLC, and Platelets counts were monitored for hematologic adverse effects monitoring, whereas, ALT, AST and bilirubin levels were monitored for efficacy. PCR was done at week 4, 12 & 36 for therapeutic success evaluation. A significant reduction in Hb levels ($p<0.05$) was observed after week 4, 8 and 12 of therapy. Frequency of anemia increased in both genders with body weight <65kg and platelet count <150,000/mm^3. End Treatment Response (ETR) was achieved in 64.5%. Anemia was the major side effect of the combination therapy particularly in the males. Higher ETR was observed in patients who achieved RVR and were <50 years of age.

Keywords: Chronic Hepatitis C, ribavirin; anemia, interferon, end treatment response.

INTRODUCTION

Chronic Hepatitis C infection is a common worldwide condition, caused by Hepatitis C Virus (HCV). This chronic infection can lead to cirrhosis with further progression to various complications including; ascites, hepatic cell carcinoma, and encephalopathy (Flamm, 2003). The available data suggested that most of the natives of the Western Europe, America, and South East Asia have an occurrence of antibody to HCV (Anti-HCV) fewer than 2.5%. Its occurrence in Eastern Europe varies from 1.5% to 5%, for the Western Pacific region from 2.5% to 4.9%, 1% for the Middle East, and more than 12 % in Central Asia. Occurrence of HCV infection in the Pakistan is 5.9% (Lavanchy, 2011).

At present no vaccine is available for HCV prophylaxis, so the focus must be towards the prevention of the spread of HCV by safe blood transfusion especially in the developing world, safe practice for use of injection in health care settings, and to decrease the number of persons who initiate the use of injectables (Shepard *et al*., 2005).

Combination of Ribavirin and Peg-interferon alpha showed strong clinical efficacy against hepatitis C, and is now documented as standard treatment (Reddy *et al*., 2008). Currently approved therapy is once weekly peg-interferon (IFN)-α subcutaneous in combination with body weight based oral Ribavirin (Naik and Tyagi, 2012). Such treatment strategies are successful in chronic patients of HCV-2 or HCV-3 infections range 75%-90%, but for HCV-1 and HCV-4 infections range from 45% and 52% (Deutsch and Hadziyannis, 2007).

Interferon and Ribavirin have synergistic action and prevent relapse of chronic Hepatitis C (Labesque *et al*., 2011). This therapy for chronic Hepatitis C has demonstrated limited success and is also associated with undesirable adverse effects (Huang *et al*., 2006). Adverse effects of interferon and Ribavirin combination therapy affect nearly all the patients with chronic hepatitis C who receive the therapy. The most frequent side effects of interferon are muscle pains and fatigue. The side effects even more difficult to handle are the psychological problems like depression, anxiety, insomnia, irritability, and difficulty in concentration (Hoofngale and Seeff, 2006). The adverse effects lead to premature termination of therapy in up to 8.7% of cases (Ogawa *et al*., 2011). Anemia is also a frequent side effect of Ribavirin in a substantial proportion of patients (Franceschi *et al*., 2000). Anemia is defined as a decrease in hemoglobin level up to 2gm/dL or more from the baseline value (Brochot *et al*., 2010). The literature suggested that there is an increased risk of anemia with every 10 years increase in age and with every 1gm/dL decrease in Hemoglobin level (Reau *et al*., 2008).

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European Association for the Study of Liver (EASL) guidelines for management of hepatitis C virus were followed for the diagnosis, goals of therapy, endpoint of therapy, treatment monitoring, treatment safety monitoring and measures to improve treatment success rates. According to EASL guidelines the response to therapy was measured in term of different parameters like Rapid Virological Response (RVR), defined as an undetectable HCV RNA at week 4 of therapy or Early Virological Response (EVR), defined as HCV RNA, which is undetectable at week 12. The goal of combination anti-HCV therapy is to eradicate HCV infection in order to prevent complications of HCV-related liver diseases and extra hepatic diseases, including fibrosis, cirrhosis, hepatocellular carcinoma and death. The end point of anti-HCV combination therapy is the Sustained Virological Response (SVR), defined by undetectable HCV RNA 24 weeks after the end of therapy while End Treatment Response (ETR) is the undetectable HCV RNA after the completion of therapy (Mutimer *et al*., 2013).

In order to reduce the chances of drug induced disorders associated with anti-HCV therapy and to maximize the desired therapeutic outcomes, effective laboratory tests and symptomatic monitoring of the patients must be carried out during treatment. This study focused on monitoring of therapy for safety and efficacy parameters and observations of this study will serve as a basis for designing of protocol for therapeutic monitoring of anti-HCV therapy in future for Pakistani population in particular so as to maximize the desired beneficial outcomes and minimize drug misadventures.

MATERIALS AND METHODS

Study design

A concurrent observational study was conducted for recording the Interferon and Ribavirin combination therapy side effects and End Treatment Response (ETR) by monitoring symptoms, laboratory reports and treatment protocol at week 4, 8 and 12 of therapy. To observe the hematological side effects levels of Hemoglobin, platelets count and TLC were monitored at week 4, 8, 12 and 16 of therapy. The levels of ALT, AST and Bilirubin were monitored for determining the efficacy of therapy at week 4, 8, 12 and 16. Polymerase Chain Reaction (PCR) for HCV RNA was performed at week 4(RVR), 12 (EVR) and 36 (ETR) of combination antiviral therapy against HCV for confirmation of HCV virus eradication and all the data were analyzed descriptively and inferential Statistics using SPSS version 16.

Selection of the patients

Inclusion criteria

Patients positive for Hepatitis C virus, both genders, 18-70 years age group, no previous treatment with Interferon or Ribavirin alone or Interferon plus Ribavirin combination therapy**,** baseline tests done were the parameters for inclusion criteria of the patients.

Exclusion criteria

Patients suffering from thyroid abnormalities and malignancies, pregnant women, patients having anemia and abnormal liver function tests, any type of liver impairment, previous history of herbal medicines use were selected as parameters for exclusion criteria.

Collection of data

Study was conducted at Hepatitis Clinic of Lahore General Hospital, Lahore, Pakistan. A total of 288 patients with chronic hepatitis C were selected who were using combination anti-Viral therapy of Interferon and Ribavirin during the months: November, 2013 to April, 2014. The dose of Interferon used was 3MU three times a week by subcutaneous injection while Ribavirin was used orally two times a day based on the weight of the patients. A data collection form has been designed having different sections for collection of information about therapeutic outcomes associated with Interferon and Ribavirin therapy in patients with chronic hepatitis C.

The data collection form was filled by researchers through verbal communication with patients, physicians and also recorded the relevant information written in the Patient Medication Record (PMR) and Laboratory Report values.

STATISTICAL ANALYSIS

Collected data were analyzed by descriptive statistics and paired T-test using SPSS version 16.

RESULTS

The data were analyzed based on different parameters like frequency of anemia, different risk factors involved in the development of anemia and response of patients towards therapy. Different predictive and risk factors for anemia include pretreatment baseline platelet count <150,000 per cmm^3, body weight <65 kilograms, age ≥50 years and female gender (Brochot *et al*., 2010). Out of 288 patients, 102 males and 115 female patients completed the duration of course of therapy for chronic hepatitis C. In rest of the 71 patients the major cause for premature termination of therapy was failure to respond to therapy (fig. 1).

The anemia was more frequently observed in males but the overall frequency of anemia increased in both genders with increase in duration of therapy for chronic hepatitis C till week 12. Highest incidence rate of anemia was observed in both male and female patients weighing less than 65kg, followed by those patients whose baseline platelet count was <150,000 per mm^3 (fig. 2). Male showed significant reduction in hemoglobin levels after week 4, 8, 12 and 16 of therapy ($p<0.05$), but in female

there was no significant reduction (table 2). Statistically significant reduction in platelet count was observed in male at 4,8,12 and 16 week of therapy but in female only after 16 week of therapy (table 2). Significant reduction in TLC was observed in both genders after 4, 8, and 12 weeks of therapy (table 2).

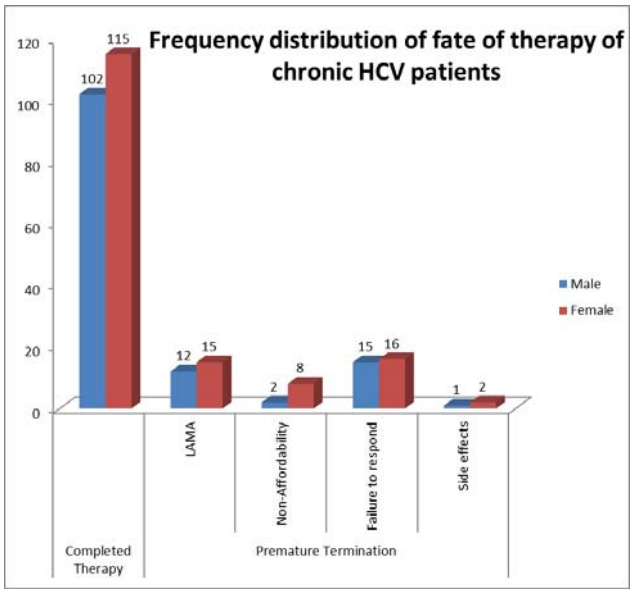

Fig. 1: Gender wise frequency distribution of fate of patients taking anti-viral therapy against HCV (n=288)

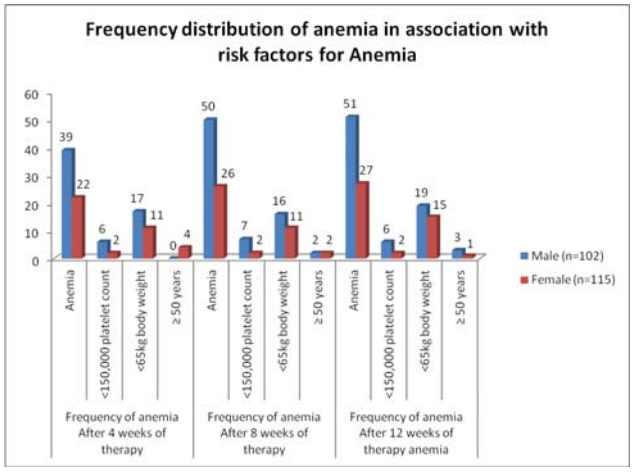

Fig. 2: Gender wise frequency distribution of anemia at week 4, week 8, and week 12 of combination therapy against HCV (Interferon plus Ribavirin) for chronic Hepatitis C and its association with different risk factors

Success of anti-HCV therapy is associated with normalization of elevated ALT, AST and bilirubin levels in HCV patients, a significant reduction in levels of ALT was observed at week 12 and 16 in males and at week 4 in females. Levels of AST were significantly reduced in males at week 12 but at week 4 in females. Levels of bilirubin were significantly reduced in males at week 16 of anti-HCV therapy (table 3).

For evaluation of efficacy of Interferon and Ribavirin combination therapy all the patients who completed the anti-HCV therapy (217) were divided into three groups depending on their age, 1st group had age less than 50 years, 2nd 50-60 years and 3rd more than 61 years. RVR and ETR were noted for all these groups. It was concluded from this study that the rate of ETR was higher in patients who achieved RVR especially in patients with age less than 50 years (fig. 3).

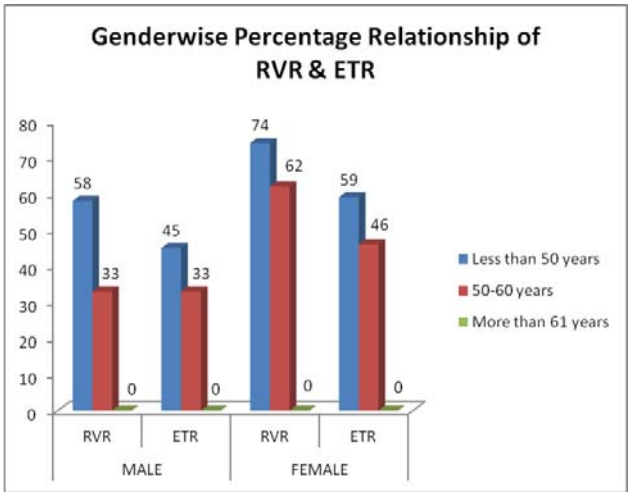

Fig. 3: Gender wise (For male n=102 & for Female n=115) percentage Rapid Virologic Response (RVR) and End Treatment Response (ETR) according to patient age group after the use of combination antiviral therapy (Interferon plus Ribavirin) against HCV

DISCUSSION

Interferon and Ribavirin combination is used to have synergistic action and prevent relapse of chronic hepatitis C (Labesque *et al*., 2009).

Failure of therapy was the major reason (43.7%) for premature discontinuation of therapy followed by left against medical advice (LAMA), non-affordability of patients for HCV RNA Polymerase Chain Reaction (PCR) test and occurrence of side effects respectively (fig. 1). Adverse effects due to combination anti-HCV therapy was observed in most of the studies as the major cause for premature discontinuation of therapy was the; 8.7% (Japan), 11% Pakistan (Peshawar), 6-13% Tokyo, 8.8% (Italy) (Ogawa *et al*. 2011; Khan *et al*., 2011; Hung *et al*., 2006; Gaeta *et al*., 2002). One reason for high rate of anti-HCV therapy failure in our study could be attributed to the use of conventional Interferon in combination with Ribavirin in contrast the EASL guidelines suggest peg-Interferon and Ribavirin as the standard treatment of chronic hepatitis C (Mutimer *et al*., 2013).

The combined anti-HCV therapy is associated with many adverse effects; the major is development of anemia and

Table 1: Clinical and laboratory population parameters of the chronic hepatitis C patients taking combination therapy of Interferon and Ribavirin as antiviral therapy

Sr. No.	Parameter	Values (Mean ±SD)
1	Mean Age (in Years)	35.15±9.03
2	Mean weight (in Kilograms)	66.19±14.13
3	Mean Platelet Count ($\times 10^3$) (in per mm^3)	244.4±74.8
4	Mean Hemoglobin (in gm/dL)	13.16±1.68
5	Mean TLC (in per mm^3)	7861.2±6779.4
6	Mean ALT (in IU/L)	58.85±44.67
7	Mean AST (in IU/L)	48.06±31.74
8	Mean Bilirubin (in mg/dL)	0.7±0.36

Table 2: Evaluation of parameters (Hemoglobin, Platelets & TLC) for the analysis of side effects of combination antiviral therapy (Interferon and Ribavirin) against HCV

	Hemoglobin (gm/dL)		Platelets count (per cubic mm)		TLC (per cubic mm)	
	Male (n=102)	Female (n=115)	Male (n=102)	Female (n=115)	Male (n=102)	Female (n=115)
Week 0 (Baseline)	14.15±1.65	12.28±1.15	234294±67232	253344±80184	7337±1727	8326±9163
Week 4	12.74±1.67	11.43±1.35	235079±93333	248739±77817	6069±1542	6201±1903
P-Value	0.000	0.017	0.008	0.707	0.000	0.000
Week 8	12.43±1.65	11.28±1.22	250000±10063	244429±80652	6161±1787	6296±1996
P-Value	0.000	0.281	0.008	0.413	0.000	0.000
Week 12	12.36±1.81	11.23±1.22	240794±81884	250921±87984	5973±1777	6188±2126
P-Value	0.001	0.168	0.000	0.372	0.000	0.000
Week 16	12.39±1.85	11.35±1.26	242930±84697	250791±78057	6183±2474	6115±2057
P-Value	0.006	0.145	0.000	0.023	0.023	0.415

Table 3: Evaluation of parameters (ALT, AST & Bilirubin) for the analysis of efficacy of combination antiviral therapy (Interferon and Ribavirin) against HCV

	ALT (IU/L)		AST (IU/L)		Bilirubin (gm/dL)	
	Male (n=102)	Female (n=115)	Male (n=102)	Female (n=115)	Male (n=102)	Female (n=115)
Week 0 (Baseline)	59.5±35.1	58.3±51.8	48.2±29.2	47.9±33.9	0.69±0.2	0.7±0.45
Week 4	40.9±16.9	41.4±30.4	37.2±17.6	35.2±17.6	0.7±0.2	0.69±0.16
p-Value	0.130	0.000	0.777	0.000	0.74	0.136
Week 8	40.4±18.4	37.01±18.07	36.5±15.3	35.05±18.58	0.7±0.2	0.7±0.24
p-Value	0.088	0.059	0.555	0.137	0.556	0.565
Week 12	39.6±19.9	43.4±19.5	34.96±17.9	35.2±19.3	0.68±0.16	0.68±0.18
p-Value	0.033	0.505	0.027	0.087	0.926	0.929
Week 16	40.4±21.8	39.04±22.7	36.59±18.8	35.1±17.02	0.7±0.36	0.65±0.17
p-Value	0.024	0.062	0.076	0.052	0.002	0.269

was observed to be more severe in Asia (Takaki *et al*., 2004). There was significant reduction in Hemoglobin levels after week 4 of therapy both in male (p=0.000) and female (p=0.017) patients but in male a significant reduction was also observed at week 8 (p=0.000) and 12 (p=0.001) of anti-HCV therapy (table 2). In both male and female patients with HCV body weight <65kg was the major risk factor for development of anemia at week 4, 8 and 12 of anti-HCV combination therapy followed by baseline platelet counts <150,000/mm^3 in male patients only (fig. 2).

Leukopenia is another side effect associated with interferon. The analysis of our study data for TLC by paired T-test also showed a significant reduction of TLC after week 4 (p=0.000), 8 (p=0.000), and 12 (p=0.000) of therapy with Interferon and Ribavirin in both male and female patients (table 2). Although there was significant reduction in leukocyte count in our study, but no patient developed Leukopenia. In a similar study conducted in Japan (Ogawa *et al*., 2011) documented that the 3% patients infected with HCV genotype 1 and 4.87% patients with genotype 2 developed neutropenia and led to

premature termination of therapy. In the same study the thrombocytopenia was observed in 3.8% patients of HCV genotype 1 and 7% patients infected with HCV genotype 2. Analysis of our study for platelets counts by paired T-tests using SPSS showed a significant reduction in platelets counts after week 4 (p=0.008), 8 (p=0.008) and 12 (p=0.000) of combination anti-viral therapy for HCV in male patients but no significant reduction was observed in females after week 4, 8 and 12 but a slight reduction (p=0.023) was observed after week 16 of combination anti-viral therapy (table 2).

Levels of ALT, AST and Bilirubin were monitored to determine the therapeutic efficacy of the combination anti-HCV therapy. Levels of ALT and AST increase as the damage to liver increases due to infection, but as the viral load decreases after using combination anti-HCV therapy the levels of ALT and AST decreases. A significant reduction in ALT level was observed after week 12 (p=0.03) and 16 (p=0.024) in males, whereas in female patients significant reduction (p=0.000) was only observed after week 4 (table 3). The levels of AST showed a significant reduction (p=0.027) after week 12 in male and at week 4 (p=0.000) in female patients. The level of bilirubin did not show any significant reduction in female patients but in males only after 16 weeks (p=0.002) of anti-HCV therapy.

Achievement of RVR is worthwhile as it provides an objective to not only improve adherence of the patient to combination anti-HCV therapy but also an indicator at which to reassess the need for continued treatment (Davis *et al*., 2003). The gender wise analysis of the relationship of ETR to RVR came up with the conclusion in our study that female and younger patients had higher rate (fig. 3). Similar results were also reported in study in which that the probability of achieving ETR was more in younger patients (Iwasaki *et al*., 2006) and higher HCV clearance rate in females compared with males (Bakr *et al*., 2006).

CONCLUSION

Failure of therapy and unaffordability were the major factors for premature discontinuation of therapy. Hematologic adverse drug events chances associated with Interferon and Ribavirin can be minimized in Chronic Hepatitis C patients be effective laboratory monitoring. Platelet counts should be also be monitored frequently in male patients receiving anti-HCV therapy as significant reduction was observed in male patients of our study. The success rate of anti-HCV combination therapy (Interferon plus Ribavirin) on the basis of ETR was 63.5%, which could have been higher with less chances of side effects if Peg-Interferon was used in combination with Ribavirin. Government should take necessary steps for provision of free of cost PCR test facilities and provision of Peg-Interferon instead conventional interferon for patients taking anti-HCV therapy.

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